# The Value of Pollen Morphology in the Classification of the Genus *Hoheria*

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*I pass with relief from the tossing sea*
*of Cause and Theory to the firm ground*
*of Result and Fact.*

- Sir Winston S. Churchill

# ABSTRACT

The primary aim of this research was to determine the value of pollen morphology in the classification of *Hoheria glabrata* and *H. lyallii*, two species of small tree which grow in the South Island mountains of New Zealand. The two taxa appear very similar in general morphology, which has led to speculation over their status as distinct species. The secondary aim was to briefly study the pollen of the remaining six taxa of the genus *Hoheria* to determine how useful pollen morphology may be for their classification. To fulfil these aims specific objectives were defined. These were to determine: (1) The level(s) at which pollen variation was significant within and between individual trees. (2) The possibility of grouping variations in pollen morphology into distinct types. (3) The way in which morphological types could be related to taxonomic schemes for the *Hoheria* genus. (4) If these pollen types could be correlated with climatic or other environmental parameters. Extensive field sampling of *H. glabrata* and *H. lyallii* pollen was carried out, while the remaining six taxa were sampled from herbarium sheets. The pollen was examined under the scanning electron microscope, where it was photographed and then measured for specific grain and spine characteristics. The data was analysed by computer using analysis of variance and discriminant analysis. Significant variation of the pollen characters was found within and between individual trees of *H. glabrata* and *H. lyallii*, but not between the two taxa. None of the eight taxa of *Hoheria* could be

separated using pollen morphological characteristics. Therefore pollen cannot be regarded as a reliable taxonomic character to differentiate either Quaternary or modern pollen of the genus *Hoheria*. Consequently the question of whether *H. glabrata* and *H. lyallii* are distinct species remains unresolved.

# ACKNOWLEDGEMENTS

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CONTENTS

# CHAPTER 1

# INTRODUCTION

## 1.1 DISTRIBUTION AND TAXONOMY OF THE GENUS *HOHERIA*

Pollen forms an important organic component in the Quaternary sedimentary strata of New Zealand. The genus *Hoheria*, common in many areas of New Zealand, is represented in the strata by spherical pollen grains with spiny ornamentation. To date *Hoheria* pollen has not been extensively studied below the genus level. However the ability to recognise pollen from individual taxa could aid in the ecological and climatic interpretation of Quaternary pollen assemblages, and in modern phylogenetic investigations of the New Zealand Malvaceae. Relationships with other New Zealand members of the Malvaceae (especially *Plagianthus*) might be elucidated from such work, as *Plagianthus* shares many gross morphological characteristics with the *Hoheria* group. The similarities suggest that these two genera may share a common ancestry. Successful identification of fossil pollen of course depends on the accurate identification of modern reference material.

The genus *Hoheria* was selected for study because its pollen is known to have extensive morphological variation. Such variation is generally considered to be unusual, as pollen is believed to be a conservative feature of plant structure.

*Hoheria* is endemic to New Zealand, distributed from North Cape to Gore, occupying inland, coastal and island habitats (Table 1). While taxa of *Hoheria* are particularly common in marginal forest environments, they also inhabit more open disturbed areas, such as river terraces and land-slips (Figure 1a).

The nomenclature of Allan (1961) is used throughout this study, with two additions which have yet to be formally described in the literature. The first is a form of *Hoheria populnea* A. Cunn. which occurs only on the Poor Knights and Hen and Chicken Islands. This taxon is referred to as *H. populnea* "Poor Knights" in this dissertation. The second form is found on the Tararua and Rimutaka Ranges, as well as the summit of Arapawa Island, and is referred to here as *H.* "tararua".

Allan recognises five species in the genus, in a taxonomic scheme which has remained unchanged since its publication in the Flora of New Zealand (Allan 1961, Table 2). Several other forms of *Hoheria*, recognised in the Flora, have not yet been formally described or named. These forms are noted under the species *Hoheria sexstylosa* Col. and *H. populnea*, the latter of which Eagle (1981) has described as "a very variable species". The term "*H. populnea*" is used throughout this dissertation to describe *H. populnea* and *H. populnea* var. *populnea*, however the *H. populnea* "Poor Knights" form is treated separately. Allan (1961) reports possible hybridization between *H. populnea* and *H. sexstylosa*, *Hoheria glabrata* Sprague et Summerhayes and *H. sexstylosa*, and *Hoheria lyallii* Hook.f. and *H. populnea*.

**Table 1 : The Distribution and Habitats of *Hoheria* (Allan 1961; Eagle 1975, 1982; Salmon 1980).**

| Taxon | Distribution and Habitat |
|---|---|
| *H. populnea* | North Island, from North Cape to the Waikato and Bay of Plenty.<br>Sea level to 450 m.<br>Lowland forest, especially margins. |
| *H. populnea* "Poor Knights" | Poor Knights and Hen and Chickens Islands<br>Coastal forest. |
| *H.* "tararua" | North Island - Tararua and Rimutaka Ranges.<br>South Island - Summit of Arapawa Island, Marlborough Sounds.<br>In forests on mountains, on riverbanks in the lowlands. |
| *H. angustifolia* | North Island and South Island, from Taranaki southwards.<br>Sea level to 900 m.<br>Lowland forest and swamp margins. |
| *H. sexstylosa* | North Island and South Island from Whangarei south to Nelson, also Banks Peninsula and near Gore.<br>Sea level to 900 m.<br>Forest and forest margins. |
| *H. sexstylosa* var. *ovata* | South Island, from North-West Nelson to Northern Westland.<br>Sea level to 1000 m.<br>In forests, especially along streams and limestone cliffs. |
| *H. glabrata* | South Island - mainly west of the main divide.<br>North Island - Mt Egmont.<br>Truly deciduous.<br>Lowland to subalpine forest and shrubland, often forming groves in avalanche paths and on slips. |
| *H. lyallii* | South Island, east of the main divide<br>Truly deciduous.<br>Forest and forest margins, shrubland, forms groves on river terraces. |

Figure 1a : *H. glabrata* amongst subalpine plants on old scree slope, Arthurs Pass.

Figure 1b : *H. glabrata* from the foot of the Temple Basin Road. Note the lobing of leaves.

Table 2 : The Taxonomy of *Hoheria*, after Allan (1961) and Druce (1987 pers. comm.).

| Allan | Druce |
|---|---|
| *H. populnea* var. *populnea* A. Cunn. | *H. populnea* var. *populnea* |
| *H. populnea* undetermined A. Cunn. | *H. populnea* "Poor Knights" |
| *H. sexstylosa* Col. | *H. populnea* var/ssp *lanceolata* |
| *H. sexstylosa* var. *ovata* Simpson et Thomson | *H. populnea* var/ssp *ovata* |
| *H. angustifolia* Raoul | *H. angustifolia* |
| *H. lyallii* Hook.f. | *H. lyallii* var/ssp *lyallii* |
| *H. glabrata* Sprague et Summerhayes | *H. lyallii* var/ssp "glabrata" |
| | *H.* "tararua" |

As a result of his field research A.P. Druce has suggested a new taxonomic scheme for the genus (A.P. Druce pers. comm., Table 2). In this classification Druce groups *H. sexstylosa*, *Hoheria sexstylosa* var. *ovata* Simpson et. Thomson, *H. populnea* var. *populnea* and *H. populnea* "Poor Knights" all under the species *H. populnea*; with each accorded varietal, or subspecies, status. This scheme also classifies *H. lyallii* and *H. glabrata* together as one species. Druce believes these two taxa to be the same species exhibiting a clinal variation, from the wetter western side of the main divide to the drier eastern side.

A new species is recognised by Druce called *H.* "tararua", and is illustrated in Eagle, 1982, where it is referred to as *Hoheria* sp. (unnamed). It appears to be sympatric with *H. sexstylosa* at the base of the Tararua Range, where these two taxa have a three month separation in flowering time (A.P. Druce, pers. comm.).

Druce also reports hybridization between *H. glabrata* and *H. sexstylosa*, as well as between *Hoheria angustifolia* Raoul and *H. lyallii*, and *H. angustifolia* and *H. sexstylosa*.

There are three close taxonomic relationships in the *Hoheria* genus:

(1) The taxa *H. glabrata* and *H. lyallii* were originally classified into the species *H. lyallii* by Hooker (1853). While they are now formally separated into two species, there have been suggestions that they may be a single species showing clinal variation (Allan 1926, A.P. Druce pers. comm., H.D. Wilson pers. comm.).

(2) *H. sexstylosa* and *H. sexstylosa* var. *ovata* are grouped by species in Allan (1961). A.P. Druce (pers. comm.) also groups these two taxa together, but as varieties, or subspecies, of *H. populnea* (Table 2).

(3) *H. populnea* "Poor Knights", while not formally described by Allan (1961), is identified as a form of *H. populnea* in the Flora of New Zealand (Allan 1961). A.P. Druce has also suggested that the "Poor Knights" form of *Hoheria* belongs within the species *H. populnea*.

I am unfamiliar with the majority of the above taxa in the field. However *H. glabrata* and *H. lyallii*, on which field studies for this research were carried out, can share a very similar leaf morphology. Such is the similarity the possibility that they are a single species exhibiting clinal variation must be considered. For example, a populaton of *H. glabrata* at the foot of the Temple Basin Road (Arthurs Pass National Park), exhibits leaf characteristics typical of *H. lyallii* from localities in Canterbury. The leaves are not crenate as is usual in *H. glabrata*, but are deeply lobed (Figure 1b), broadly ovate and chartaceous (c.f. Allan, 1961), as is normal in *H. lyallii*.

This population of *H. glabrata* occurs in an exposed zone of subalpine scrub. The trees appear stunted, with numerous thick, short branches. To the east and west of this location populations of more 'typical' *H. glabrata* occur along sheltered forest margins. The Temple Basin location may well be compared to open subalpine sites along the eastern ranges of the main divide which support more

'typical' populations of *H. lyallii*. The population of *H. glabrata* at Temple Basin seems to be intermediate in morphology between this species and *H. lyallii*.

## 1.2 POLLEN VARIATION IN OTHER TAXA

The concept of variations in pollen size and morphology within similar plant species was introduced by Darwin in 1877 (Chinnappa and Warner 1982). Since then, and more specifically since the development this century of both scanning and transmission electron microscopes, variations in pollen morphology within and between taxa have been examined in great detail. Studies of modern and fossil pollen have mostly concentrated on morphological variation and taxonomy, (Olsson 1975, Clarke 1978, Miyoshi 1983, Simpson 1983, Zavada 1983, Raj and Grafström 1984 and Díez, Valdés and Fernández 1986) polymorphism (McNeill and Crompton 1978 and Chinnappa and Warner 1982) and pollen description (Coetzee and van der Schijff 1979 and Christensen 1986).

Those studies concentrating on pollen morphology have discovered variations in grain size and shape, number of apertures and exine ornamentation. Pollen grain size has been found by many authors to exhibit intraspecific variation as great as, or greater than, interspecific variation. Ritchie Bell (1959) discovered this to be true for petunia, Harris (1965a) for *Nothofagus* and Olsson (1975) for *Quercus*. Kuprijanov (1940, in Ritchie Bell

1959) found pollen size to vary from anther to anther on individual tomato, egg-plant, onion, carrot, watermelon, sunflower and wheat plants. Many interacting environmental variables appear to affect grain size, as discussed briefly in Section 4.1, and in more detail in Muller 1979. However it appears to be difficult to distinguish the particular environmental variables which have the greatest effect on pollen grain size (Jones and Newell 1948, Mikkelsen 1949 and Ritchie Bell 1959).

While some authors found pollen variation not to support present taxonomic relationships (Ackerman and Williams (1980) for the tribe Neottieae (family Orchidaceae), Raj and Grafström(1984) for the ten genera of the family Chloanthaceae, and Díez, Valdés and Fernández (1986) for four genera of the Boraginaceae) others found it most useful for this purpose (Clarke (1978) for generic relationships in the Valerianaceae and Sáenz de Rivas (1979) for 36 Spanish species of Cistaceae). Miyoshi (1983) took the process of pollen variation and taxonomic identification one step further when he related the modern pollen of the two varieties of *Castanopsis cuspidata* to fossil C. *cuspidata* pollen. The subtle variation of exine ornamentation, which distinguishes the two varieties of modern C. *cuspidata*, is also present in the fossil pollen of this species.

Pollen polymorphism is known from a number of families, the Rubiaceae being the most complex family where pollen dimorphism occurs in 68 of the genera (Vuilleumier 1967, in Chinnappa and Warner 1982). McNeill and Crompton (1978) discovered pollen dimorphism in male plants of

*Silene alba*. The pollen they examined had two basic types of ektexine which appeared to be associated with differences in grain diameter, wall thickness and pore number. Out of 38 species and varieties of *Coffea* (Rubiaceae) surveyed, Chinappa and Warner (1982) found 13 to have di-, tri-, tetra-, penta- and polymorphic pollen morphologies. Christensen (1986) observed dimorphic spines in a number of species from the Malvaceae. However, like many authors reporting polymorphism no attempt was made to explain the cause(s) of this feature.

Pollen morphology of 120 species from 40 genera of the Malvaceae have been broadly described by Christensen (1986). Coetzee and van der Schijff (1979) studied the South African Malvales, describing the quantitative and qualitative characteristics which they found useful for numerical analysis and keying out species. Descriptive studies such as these are most useful for the classification of taxa, especially in the case of Coetzee and van der Schijff (1979) where the characters used for the construction of a key are listed.

## 1.3 THE ROLE OF NUMERICAL METHODS IN POLLEN IDENTIFICATION

Numerical methods have been widely applied to the identification of pollen grains. Cain and Cain (1948), Mack (1971) and Ting (1966) have used numerical methods for studying *Pinus*, Birks and Peglar (1980) for *Picea*, Olsson (1975) for *Quercus*, Coetzee and van der Schijff (1979) for

the Malvales and Harris (1965a) for *Nothofagus*.

Numerical methods are particularly useful for the identification of pollen from Quaternary strata. Gordon and Prentice (1977) comment that numerical analysis based on the identification of a single grain may be unsatisfactory. However analysis involving the fitting of frequency histograms using modern pollen data, to observe frequency histograms of fossil material, may prove satisfactory. Birks and Peglar (1980) have successfully used discriminant analysis on size data from modern *Picea* pollen to distinguish three species reasonably well. They then applied these methods to Quaternary *Picea* pollen which also resulted in apparently successful identifications.

However, numerical analysis should be approached with some caution. While such analyses introduce a degree of objectivity, they are only as accurate as the data on which they are based. Individual characters may give anomalous results. For example grain size and shape of fossil pollen may alter with the compaction associated with sedimentation, or in modern pollen grain size and shape may alter for a variety of ill-defined reasons (Jones and Newell 1948, Mikkelsen 1949, Ritchie Bell 1959). Both preparation technique and mounting media are known to affect size and shape of pollen (Faegri and Iversen 1975).

Studies of modern pollen for comparison with Quaternary pollen must assume that intraspecific variation in past pollen spectra is much the same as today. Such studies must also assume that evolution since the time of pollen deposition has been insignificant (Birks and Peglar 1980).

## 1.4 AIMS AND OBJECTIVES

The primary aim of this research was to determine the value of pollen morphology in the classification of the two taxa *H. glabrata* and *H. lyallii*. It was hoped that the pollen of these two taxa could be separated according to morphological type. These taxa were chosen for this investigation for two reasons:

(1) It has been suggested that these two taxa are actually one species exhibiting clinal variation (Allan 1926, A.P. Druce pers. comm., H.D. Wilson pers. comm.). It was hoped that through the use of numerical methods the pollen might be used to verify this idea.

(2) The distribution of these two species, mostly in Canterbury and Westland, permitted intensive 'within' and 'between' tree sampling to be done.

The secondary aim of this research was to determine the value of pollen morphology in the classification of all eight taxa of the genus *Hoheria*.

To fulfil the aims of the study four specific objectives were defined. These were to determine:

(1) The 'level(s)' (spine, grain, anther, flower, branch, tree) at which pollen variation was significant within and between individual trees.

(2) Whether variations in pollen morphology could be grouped into distinct types.

(3) How these morphological types were related to taxonomic schemes for the *Hoheria* genus.

(4) Whether the pollen morphological types were correlated with climatic or other environmental parameters.

## 1.5 SUMMARY OF INVESTIGATIONS

Four separate investigations were carried out to fulfil the aims and objectives of this study:

(1) The light microscope study. This study examined the *Hoheria* genus to confirm whether there was excessive variation in its pollen morphology (Section 2.2.2.2).

(2) The initial investigation of *H. glabrata* and *H. lyallii*. To ascertain the nature of the variation and accurately quantify it, a detailed analysis of interspecific and intraspecific pollen diversity was necessary. To accomplish this four trees of *H. glabrata* and *H. lyallii* were intensively sampled from two populations (Section 2.2.1.2). The magnitude of pollen morphological variation, at all levels within the sampled population, was determined by analysis of variance (Section 2.4.2.2). This was followed by a discriminant analysis which used the same data to attempt a species separation (Section 2.4.2.3).

(3) The major *H. glabrata*-*H. lyallii* analysis. On completion of the above investigation 20 more trees from these two taxa were sampled, processed and analysed by discriminant analysis (Sections 2.2.1.3, 2.3.1 and 2.4.3 respectively). The purpose of this analysis was to try to separate the sampled trees into two taxa using selected pollen characters (Section 2.1).

(4) *H. populnea* et al. study. Following the above investigations a brief study of all the taxa of the genus was carried out (Section 2.2.2.3, 2.4.4). As the samples were collected from herbarium sheets, intensive

intraspecific analysis was not possible. Assumptions concerning the magnitude of variation at each level sampled within the taxa were made from the results of the initial *H. glabrata-H. lyallii* investigation (Section 3.1). This investigation consisted of four separate discriminant analyses (Section 2.4.4) set up to 'best' separate these species using pollen morphological characters.

# CHAPTER 2

# METHODS AND MATERIALS

## 2.1 SELECTION OF POLLEN CHARACTERS

### 2.1.1 Introduction

Individual characters, or sets of characters in combination, were used to determine the significance of pollen variation within and between the taxa of *Hoheria*. These characters were derived from the spiny sculptured exine of the pollen grains, and from the shape of the grains (Figures 2a and 2b). Consistent and accurate measurement of the characters was therefore critical to the subsequent statistical analysis (Section 2.4). Consequently characters requiring a subjective description, such as spine shape, were avoided.

Scanning electron micrographs of the pollen grains, magnified approximately 1500x (Section 2.3.3) were used for making all measurements. The scanning electron microscope (SEM) was used in preference to the light microscope due to the much greater magnifications possible. This allowed closer observations of surface detail, and therefore increased the accuracy of all measurements. The SEM also has a greatly increased depth of field compared to the light microscope, and consequently many more features can be observed in focus from a single electron micrograph.

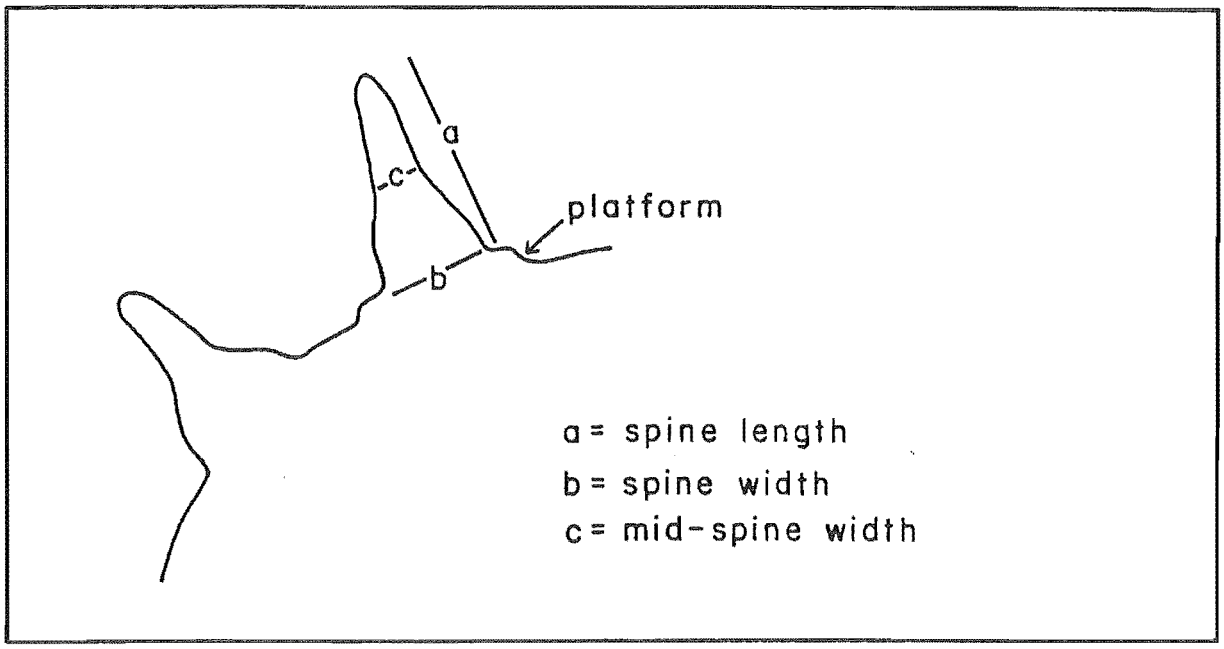



Figure 2a : Schematic diagram of a pollen grain showing spine characters.

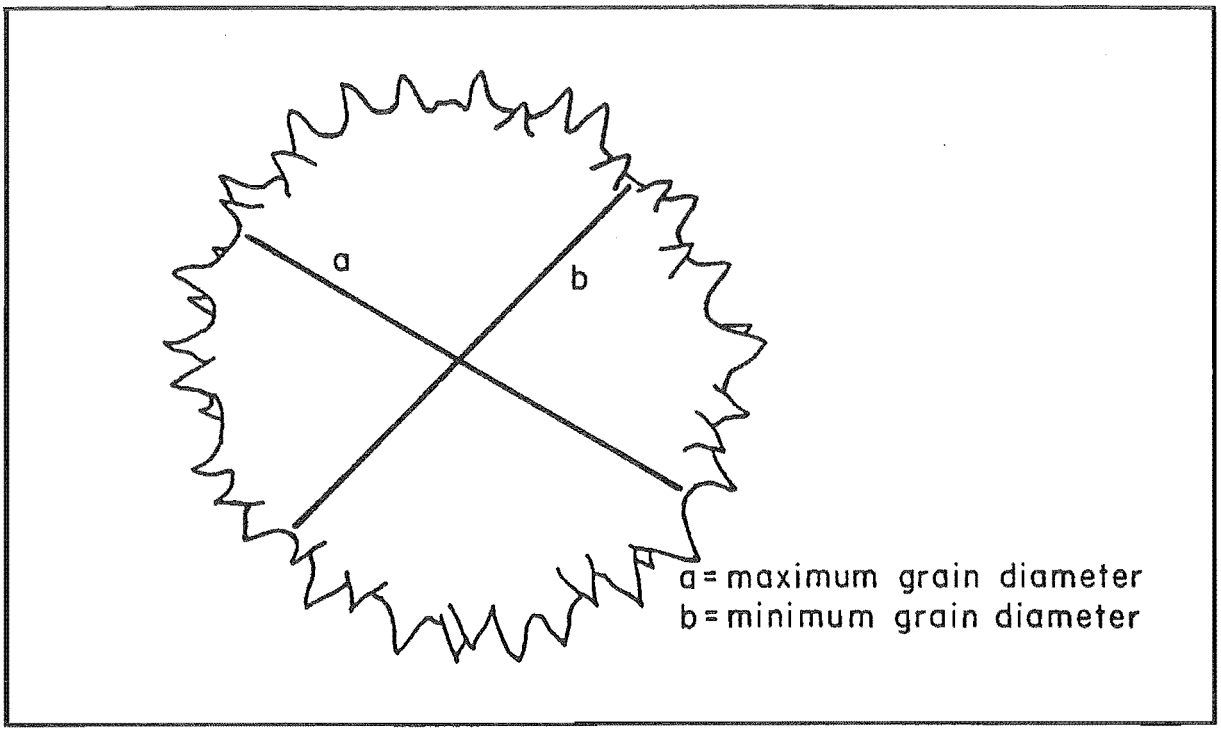



Figure 2b : Schematic diagram of a pollen grain showing grain characters

### 2.1.2 Character Synopsis

The computer program classified individual spines into the appropriate taxonomic group, based on the individual's spine and grain characteristics. Therefore spines were the basic unit of classification. For the initial investigation of *H. glabrata* and *H. lyallii* nine pollen characters were selected (Figures 2a and 2b):

(1) Spine length - the measurement from the tip of the spine to its base. The spine may enter the exine directly or it may be seated on a platform. The platform was excluded from this measurement.

(2) Spine width - the measurement across the base of the spine, excluding the platform if present.

(3) Mid-spine width - the measurement of spine width at halfway along the length of the spine.

(4) Spine taper - the spine length measurement divided by spine width.

(5) Spine taper rate - the mid-spine width measurement divided by spine width at the base.

For each pollen grain three spines were measured for the five spine characters listed above. All spines were measured with their long axis in the plane of the screen.

(6), (7) Maximum and minimum pollen grain diameters - these were measured on grains in polar view, where the grains appeared approximately spherical, to estimate pollen size. Diameter measurements excluded spines (Figure 2b).

(8) Grain ellipse - the maximum grain diameter divided by the minimum grain diameter.

(9) Spine density - three different methods of estimating spine density were tested using electron micrographs of six pollen grains. Each grain selected was approximately equal in size, with a similar number of spines. The method which produced the least variable estimates of density would be used in the subsequent analyses.

(i) The Hemisphere Method.

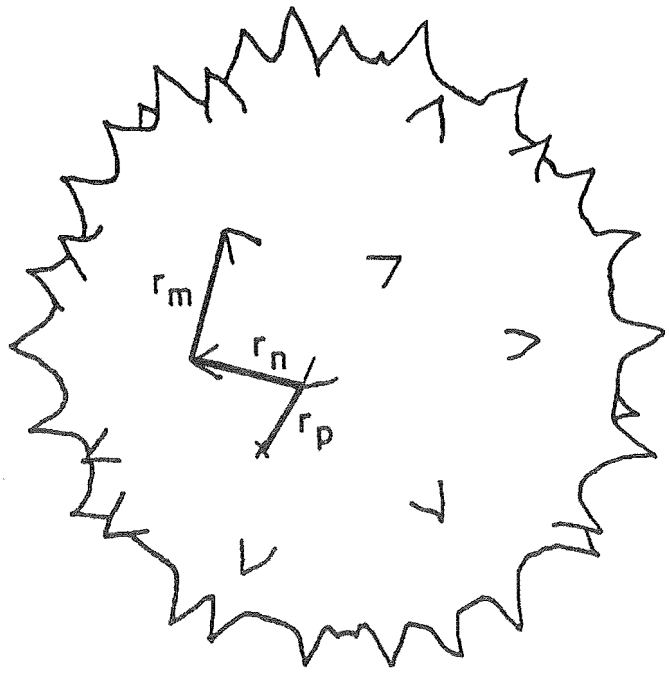
All spines on the visible hemisphere of each pollen grain were counted from an electron micrograph. This number was then divided by the area of a half-sphere $(4\pi r^2)/2$, the radius of which was calculated as

$$\frac{\text{maximum diameter + minimum diameter}}{4}$$

to give an estimate of spine density. In this way an average radius was calculated for each grain. Unfortunately many pollen grains, in both the initial and major *H. glabrata-H. lyallii* analyses, had partially sunken into the double-sided sellotape on the SEM mounts (Section 2.3.1). Therefore this method could not have been used on all grains.

(ii) Batchelor's Nearest Neighbour Method.

Six randomly spaced points were marked on the central area of each pollen grain. The distance from each random point to the nearest spine tip was then measured, then from that spine to its nearest neighbouring spine, and then from the second spine to its nearest neighbour spine (Figure 3). The distance measurements were then entered into Batchelor's formulae (Batchelor 1971, 1973, 1975) to obtain density estimates. Each estimate was then multiplied by a correction factor (Figure 4) which allowed for the greater diameter of the sphere occupied by the spine tips (the



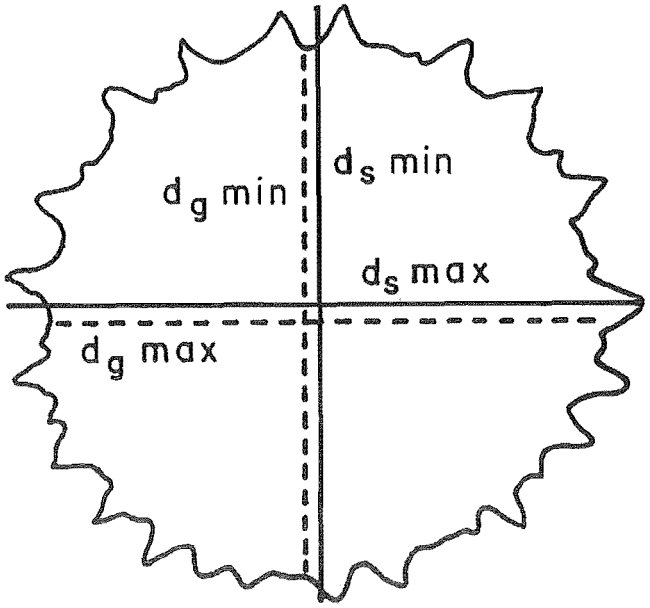

$r_p$ is used to determine the biased density estimate

$r_n$ is used to estimate first order clumping

$r_m$ is used to estimate second order clumping

x = random point

Figure 3 : Batchelor's method of estimating spine density (Batchelor 1971, 1973, 1975).




$d_g$ min = minimum diameter, excluding spines

$d_g$ max = maximum diameter, excluding spines

$d_s$ min = minimum diameter, including spines

$d_s$ max = maximum diameter, including spines

$$\overline{d_s} = d_s \text{ min} + d_s \text{ max} / 2$$

$$\overline{d_g} = d_g \text{ min} + d_g \text{ max} / 2$$

$$\text{Correction factor} = \overline{d_s}^2 / \overline{d_g}^2$$

Figure 4 : The correction factor for Batchelor's nearest neighbour technique and the half-radius method of estimating spine density.

points of measurement) to that occupied by the spine bases.

(iii) The Half-Radius Method

On each of the six pollen grains, a circle with a radius equal to half the radius of the grain in polar view was drawn. The number of spine tips in each circle was then counted and divided by the area of the circle, $\pi r^2$, to give a density estimate (Figure 5). This figure was then multiplied by the correction factor, as set out in Figure 4, for the same reason as given above.

The density estimates, resulting from the three methods were then compared (Figure 6). Overall the Half-Radius Method was the least variable, and was therefore selected for all subsequent analyses.

Two other characters, pore size and spine platforms, were not included in the analysis. Pores are not visible in fresh pollen, being covered by a membrane until the grains are acetolysed. Once acetolysed they are still difficult to observe under the SEM. Unless they appear in the centre of the grain they tend to be partially or fully concealed by spines, veruccae or undulations in the exine surface, as shown in Figure 15. Pores were therefore not measured or counted on pollen grains prepared for the SEM. However they were clearly visible on grains acetolysed for light microscope study. Here the grains appeared transparent and it was possible to focus through the grain, accurately counting the number of pores present. A rapid examination of 100 grains showed *H. lyallii* and *H. glabrata* to have four to six pores per grain, while the remaining six taxa of *Hoheria* usually had three to five pores per

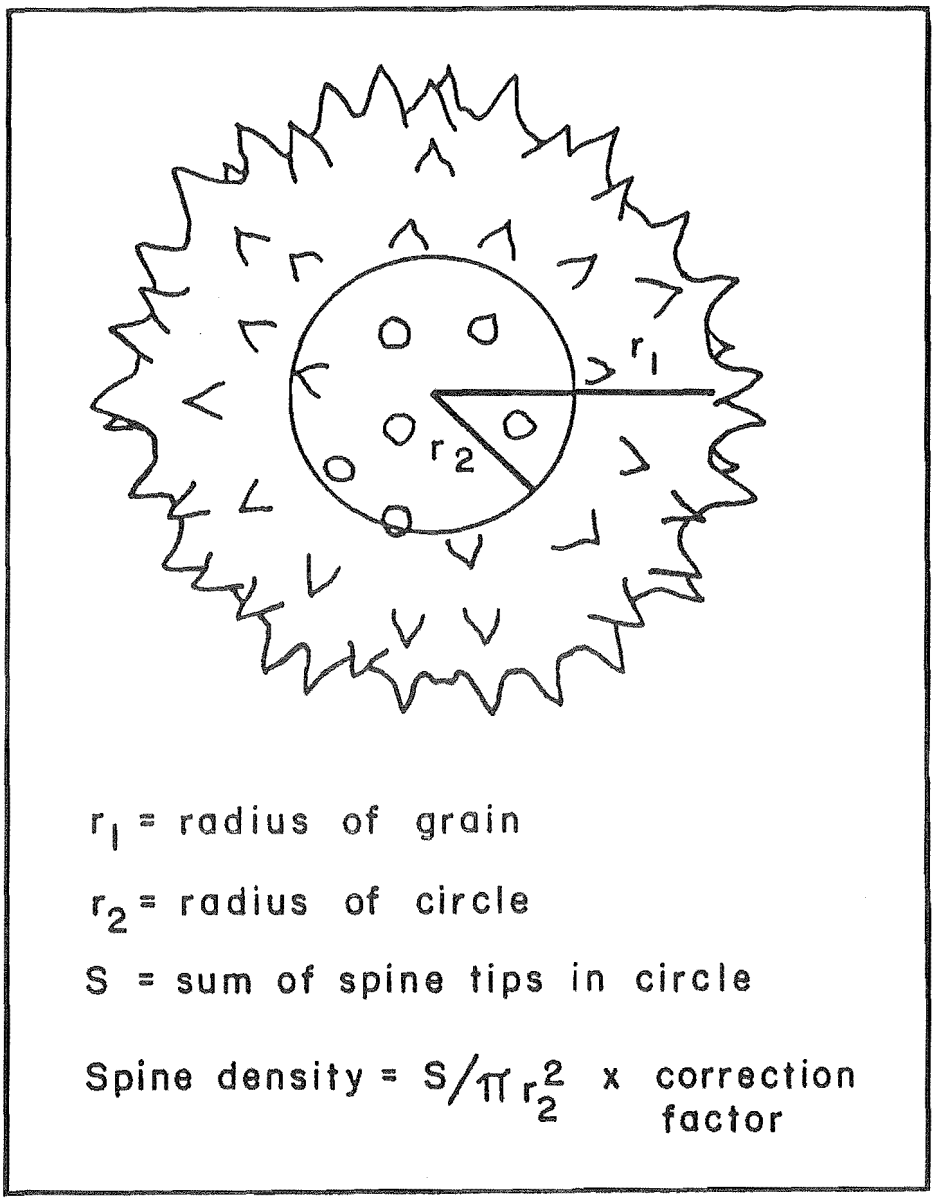
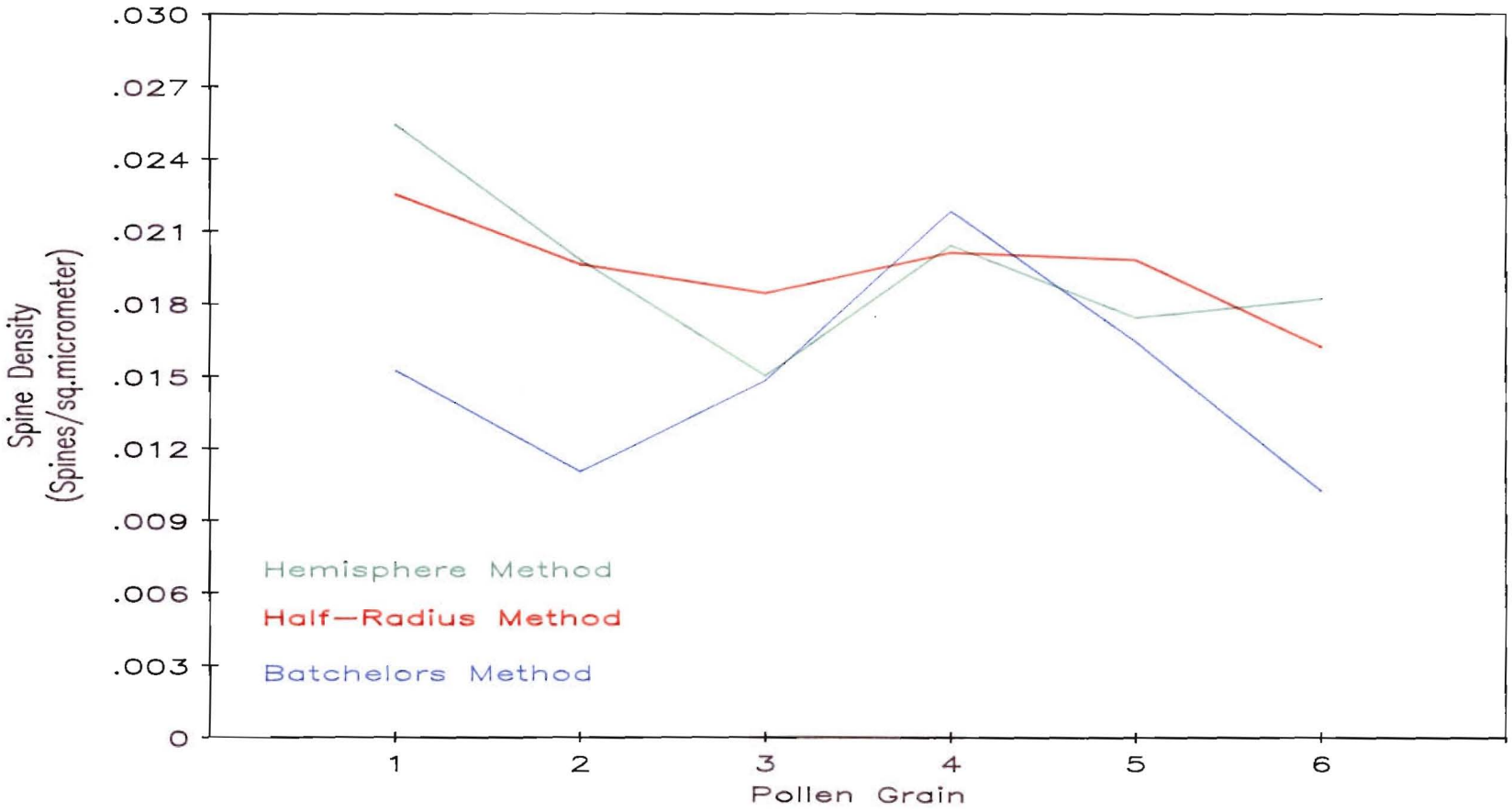



Figure 5 : The half-radius method of estimating spine density.

Figure 6. Comparison of Three Methods for Estimating Spine Density.



grain. Pore size is approximately equal throughout all taxa.

Spine platforms were frequently difficult to measure accurately. While some platforms could be easily distinguished from the exine, many others graded into it. Platforms were not present on all grains, but were either present under all spines within a grain, or absent from the grain (Figures 7a and 7b). Single anthers produced grains with and without platforms in both *H. glabrata* and *H. lyallii*. There appeared to be no trend to their presence, either within or between species. For these reasons platforms were not used as a character in this study.

## 2.2 SELECTION OF POLLEN SAMPLES

### 2.2.1 Fresh Pollen Collection

#### 2.2.1.1 Introduction

Fresh pollen, removed from trees on site, was used for the initial and major investigations of *H. lyallii* and *H. glabrata* (Figures 7a and 8a). All samples were collected from sites in Canterbury and Westland during the months of January and February 1986 (Figure 9, Appendix 1). Sites were chosen to give a good geographic spread, and on the basis of pollen availability, with individual trees being selected according to flower maturity. Pollen was removed from newly opened 'cup-shaped' flowers in which the anthers had only recently dehisced. At this stage pollen grains are mature and therefore fully developed in both size and shape; the flowers are also less likely to have

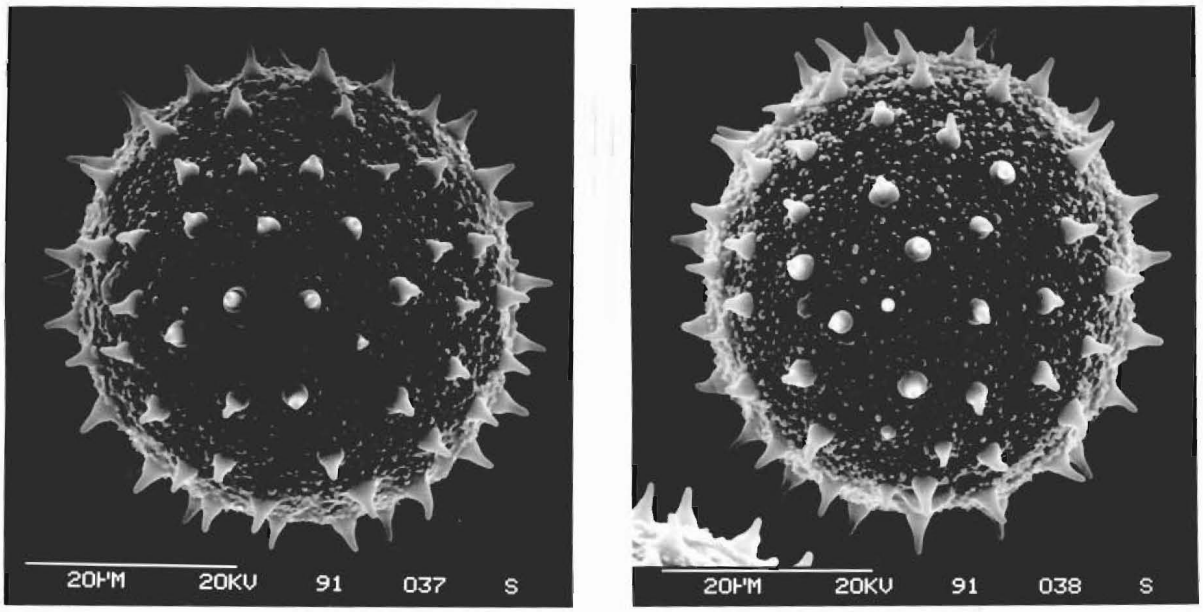



Figure 7a : Scanning electron micrographs of fresh pollen grains of *H. lyallii*.

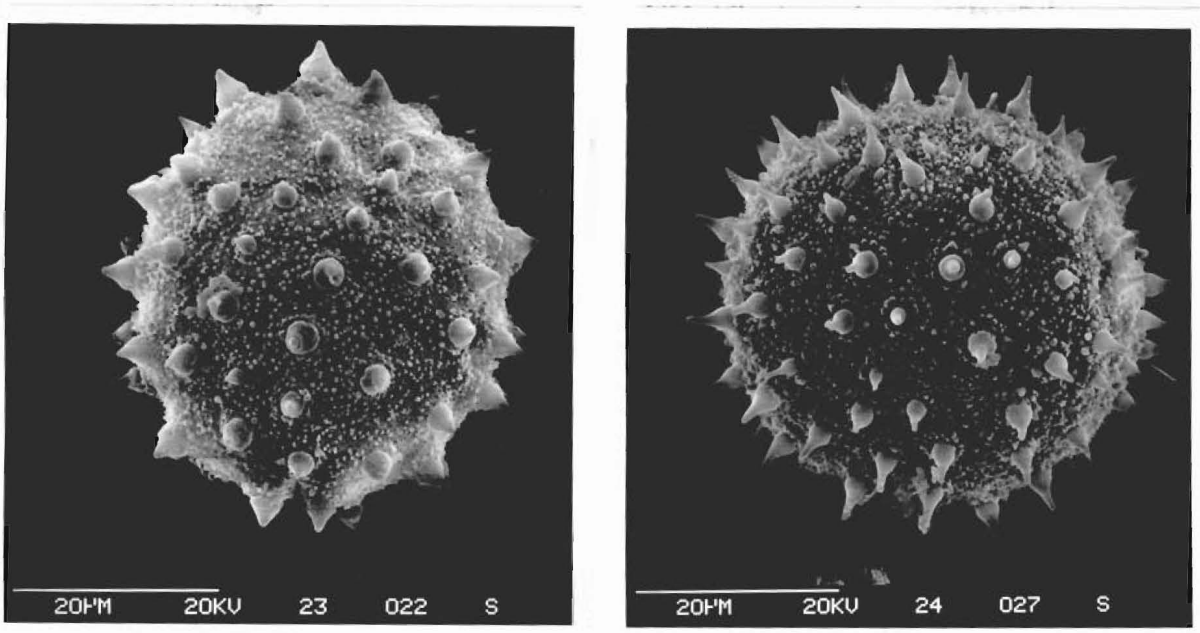



Figure 7b : Scanning electron micrographs of acetolysed pollen grains of *H. lyallii*.

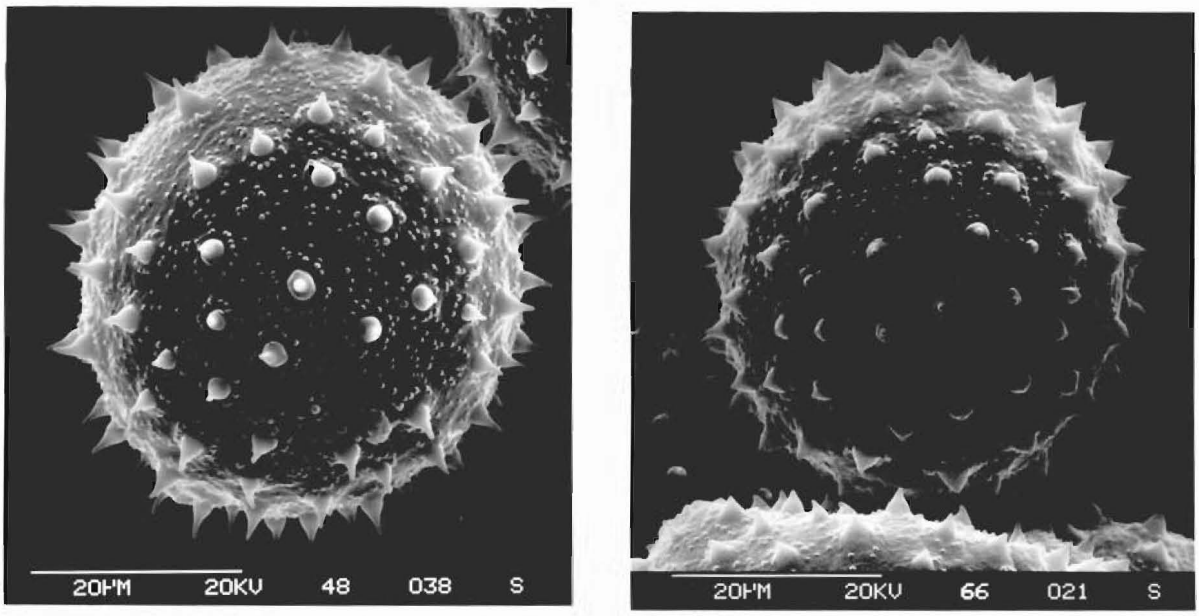



Figure 8a : Scanning electron micrographs of fresh pollen grains of *H. glabrata*.

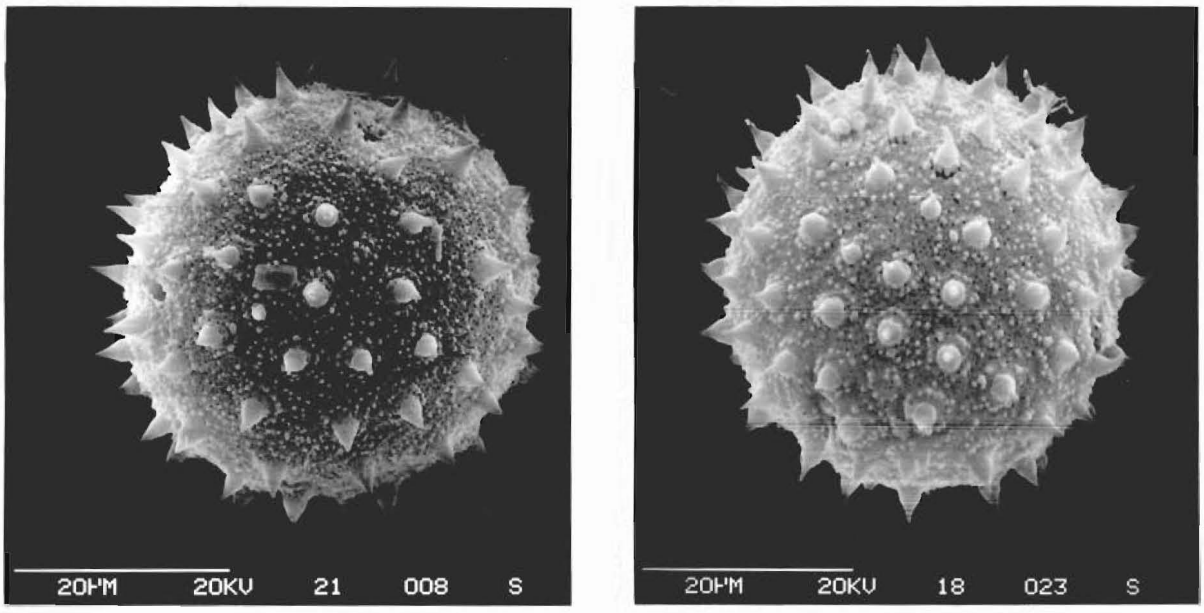
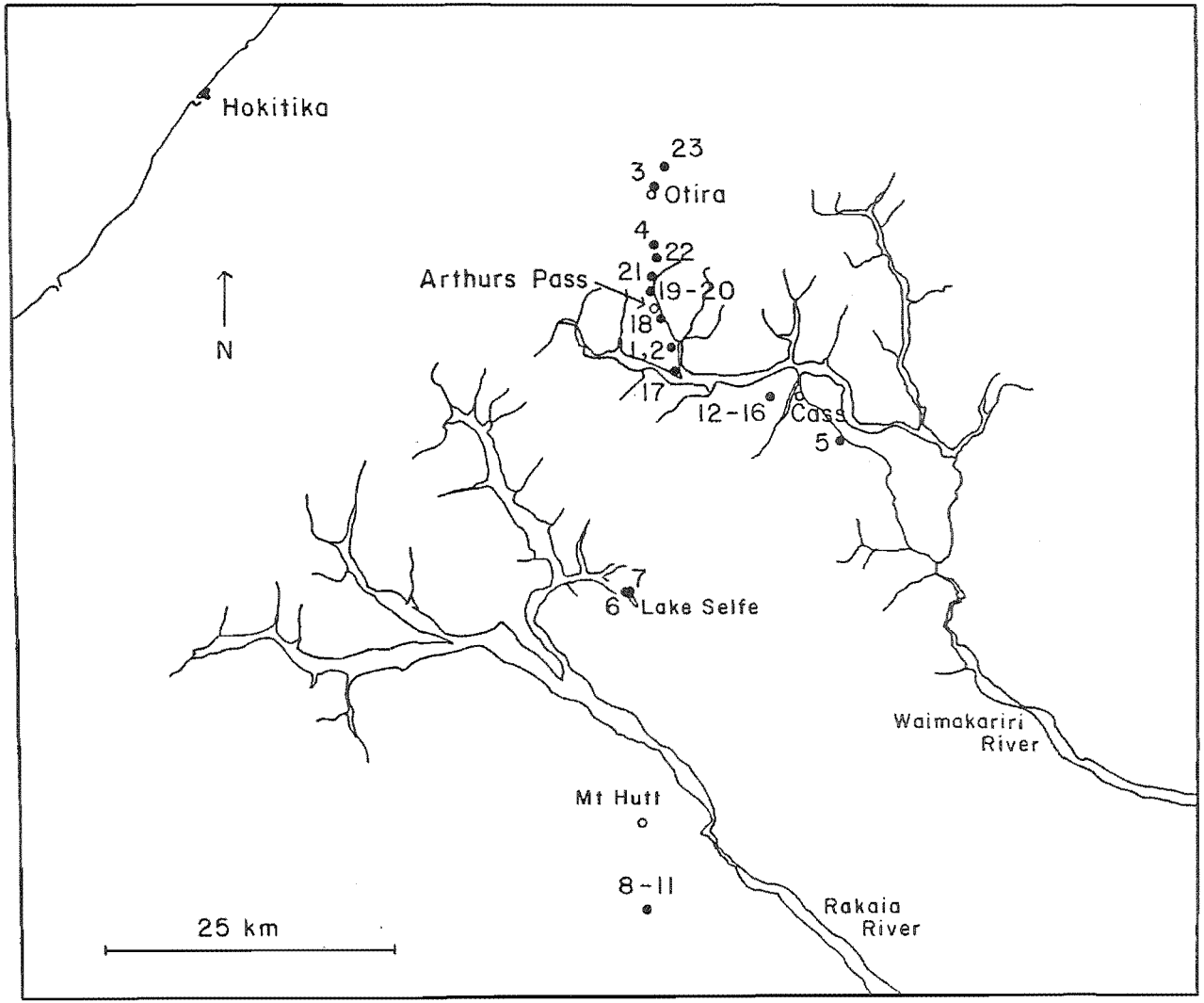



Figure 8b : Scanning electron micrographs of acetolysed pollen grains of *H. glabrata*.




Legend

| Site No. | Location |
|---|---|
| 1,2 | Greyneys Flat |
| 3 | 150 m N. Otira |
| 4 | Bottle Flat |
| 5 | Ribbonwood Stream |
| 6,7 | Lake Selfe |
| 8-11 | Awa Awa Rata Reserve |
| 12-16 | Pylon Gully |
| 17 | Klondyke Corner |
| 18 | South side Arthurs Pass township |
| 19,20 | Foot of Temple Basin Road |
| 21 | 1 km N. Dobson Memorial |
| 22 | Pegleg Creek |
| 23 | 6 km N. Otira |
| 24 | Homer Tunnel, Fiordland (not shown) |

Figure 9 : Location of field sites for collection of *H. glabrata* and *H. lyallii* pollen in Canterbury and Westland, South Island, New Zealand.

attracted insects depositing foreign pollen. Whole anthers were removed from flowers at each site, placed in separate paper bags and retained for processing in the laboratory. Paper bags were preferred to plastic as plastic promotes fungal growth. In most cases anthers were stored in the paper bags for less than 36 hours, with the longest storage time being 72 hours.

#### 2.2.1.2 Initial Investigation of *H. glabrata* and *H. lyallii*

The initial investigation was done to determine the most efficient sampling strategy for the major *H. glabrata*-*H. lyallii* analysis. Its purpose was to determine at what level the most significant variation existed within and between trees (that is comparing spines, grains, anthers, flowers, branches and trees) and to adjust the number of replicate samples accordingly.

For this analysis one pollen grain per anther for *H. glabrata* and three pollen grains per anther for *H. lyallii*, were measured for the nine pollen characters listed in Section 2.1.2. More measurements were taken but not all could be used in the analysis. Two anthers per flower were removed from two flowers per branch, with two to three branches per tree being sampled. Due to the early flowering of *H. lyallii* at the field site, good pollen samples were obtained from only two branches per tree; while three branches per tree were sampled for *H. glabrata* (Figures 10a and 10b). In total four trees, two from each taxon, were selected from two sites considered typical of the habitats occupied by *H. glabrata* and *H. lyallii*. The

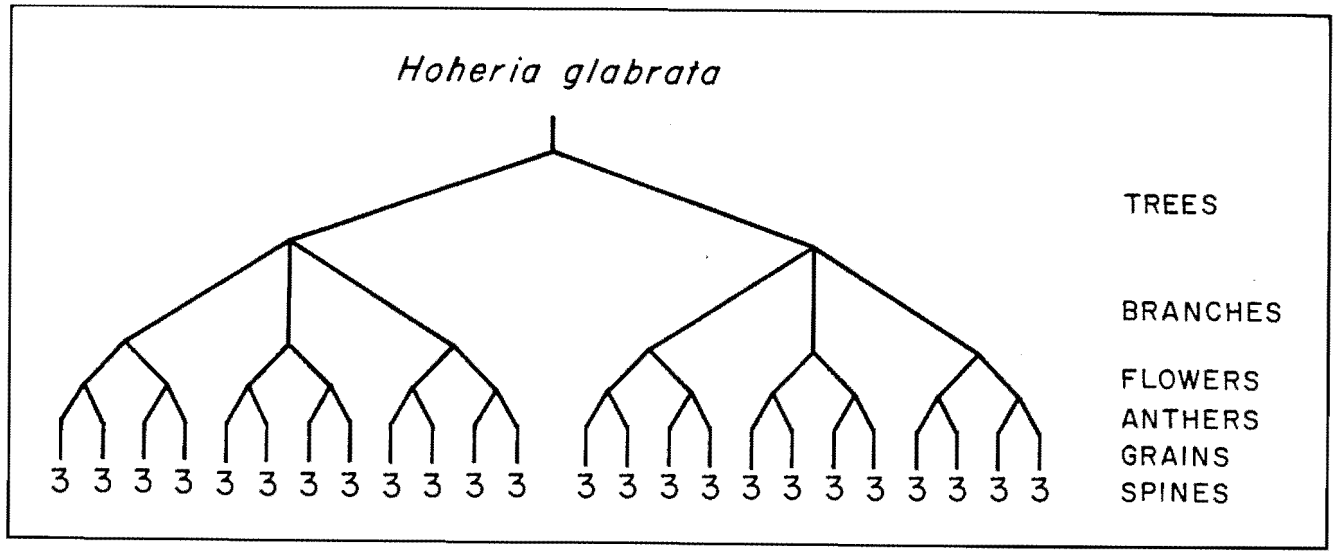
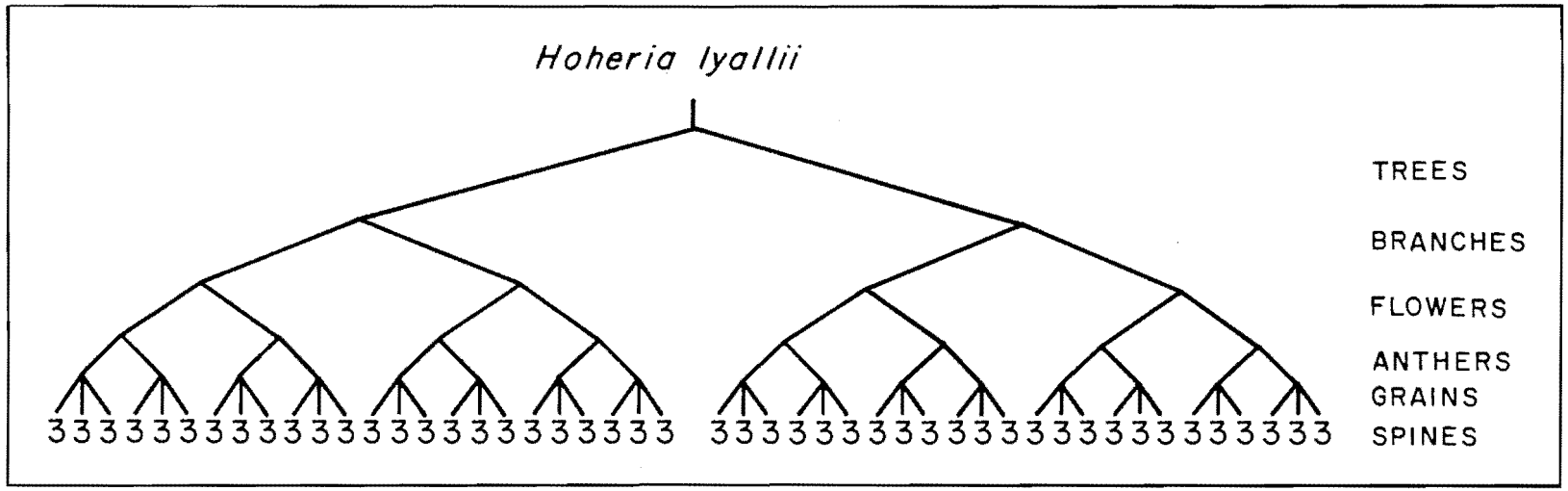



**Figure 10a : Sampling strategy for *H. glabrata* in the initial investigation.**

**Figure 10b : Sampling strategy for *H. lyallii* in the initial investigation.**



sites were at Arthurs Pass and Mount Hutt respectively (Figures 11 and 12). Typical flowers and leaves of these taxa are shown in Figure 13.

As a result of the initial investigation (Section 3.1) the sampling method was modified for the subsequent investigations. For these analyses seven of the original nine characters from each pollen grain were selected for measurement, and the number of grains sampled greatly reduced for the major *H. glabrata-H. lyallii* analysis.

#### 2.2.1.3 Major *H. glabrata-H. lyallii* analysis

Following the results of the initial investigation 24 trees were sampled, 12 of each species, from 14 different localities (Figure 9, Appendix I). This number included the four trees of the initial investigation. For this analysis six grains per anther from two anthers per tree were sampled, the samples being removed from any part of the tree (Figure 14).

### 2.2.2 Pollen from Herbarium Collections

#### 2.2.2.1 Introduction

Both the light microscope study and the *H. populnea* et al. study using the SEM were based on acetolysed herbarium material (Figures 7b, 8b, 15-20). This material came from collections held by the Botany Division, D.S.I.R. Herbarium and the Auckland Institute and Museum Herbarium (Appendix II). The samples were acetolysed to remove debris adhering to the grains.

Figure 11a : Awa Awa Rata Reserve at the base of Mt Hutt. Site of pollen collection for *H. lyallii* used in the initial investigation.

Figure 11b : *H. lyallii* tree, sampled at the Awa Awa Rata Reserve, Mt Hutt.

Figure 12a : Greyneys Flat, Arthurs Pass National Park. Site of pollen collection for *H. glabrata* used in the initial investigation. (Pollen collected from trees behind the shelter).

Figure 12b : *H. glabrata* tree, sampled from the southern side of Arthurs Pass township.

Figure 13a : *H. lyallii* in flower, Ribbonwood Stream.

Figure 13b : *H. glabrata* in flower, Halpins Creek, Arthurs Pass National Park.

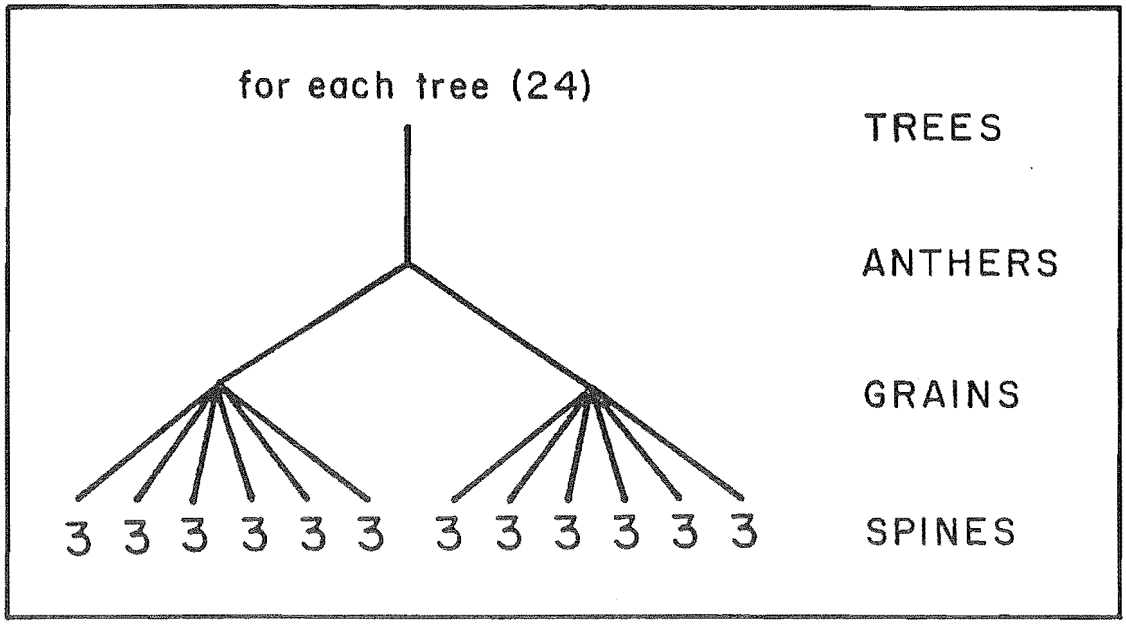



Figure 14 : Sampling strategy for the 24 trees of the major *H. glabrata-H. lyallii* analysis.

2.2.2.2 Light Microscope Study

The specific aim of the light microscope study was to confirm by observation the existence of pollen variation in the genus *Hoheria*. Consequently complex sampling procedures were unnecessary. In total 20 whole flowers, representing the eight taxa of *Hoheria*, were removed from separate herbarium sheets. All samples were acetolysed and mounted in glycerine jelly on reference slides (Section 2.3.2.1) for study under a light microscope. Pollen measurements were not made from these slides due to the preliminary nature of this investigation. However, considerable variation was observed in pollen grain size, and spine shape and density. This prompted the full investigation into the genus *Hoheria*.

2.2.2.3 *H. populnea* et al. Study

A limited pollen study of all the taxa of *Hoheria* was undertaken to indicate the magnitude of variation within the genus. Two pollen samples from separate herbarium sheets were selected from each of the eight taxa except for *H. sexstylosa* and *H. sexstylosa* var. *ovata*, where four and five pollen samples respectively were collected (Appendix II). These latter two taxa were sampled more extensively to determine the extent of intra-taxon variation.

Pollen from the type specimens of *H. populnea*, *H. glabrata* and *H. lyallii* was requested from Kew Gardens, England. Unfortunately there is no pollen remaining on the flowers of these specimens. The two samples each of *H. glabrata* and *H. lyallii* used in this analysis were

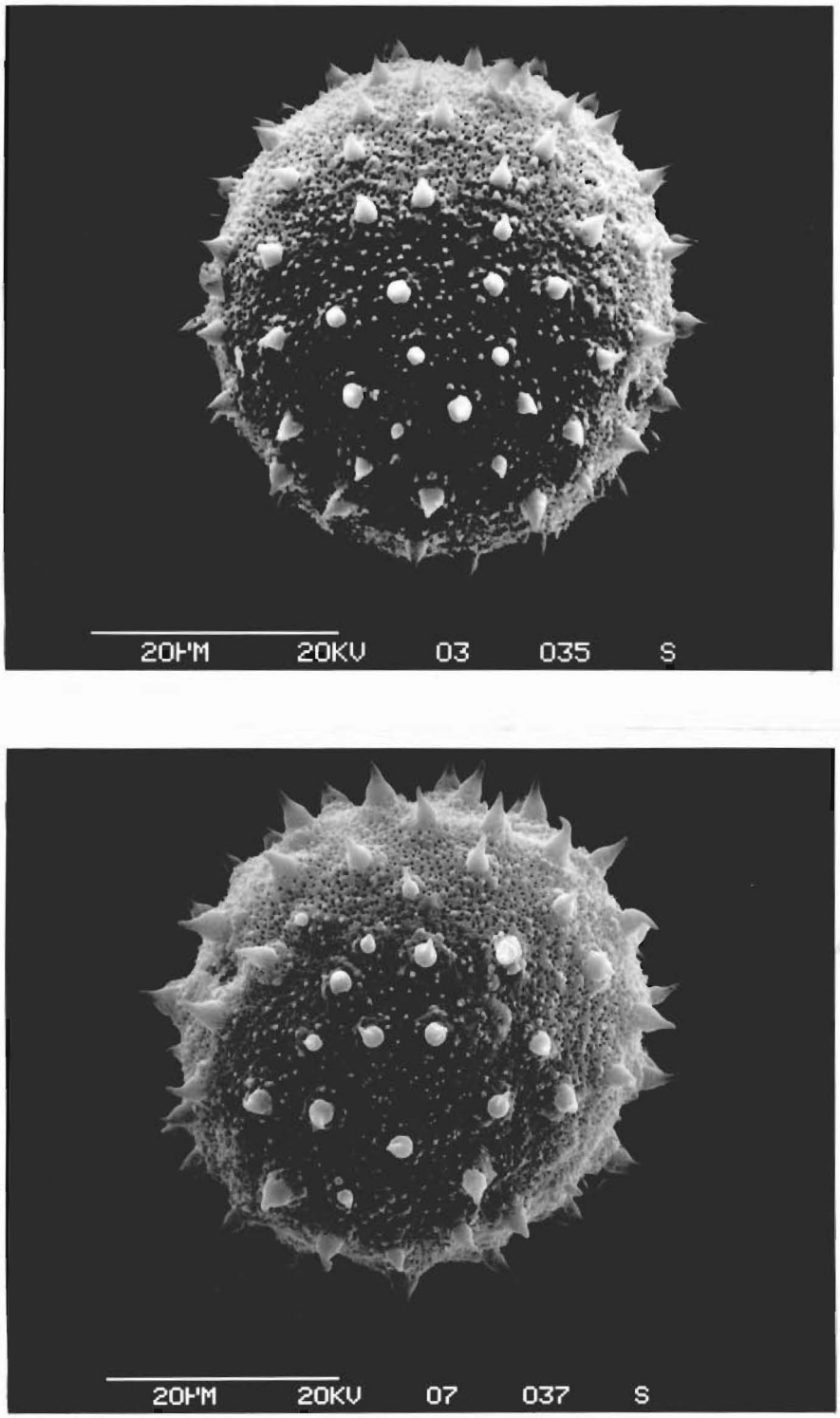



Figure 15 : Scanning electron micrographs of acetolysed pollen grains of *H. sexstylosa*. Note the partially concealed pores in both micrographs.

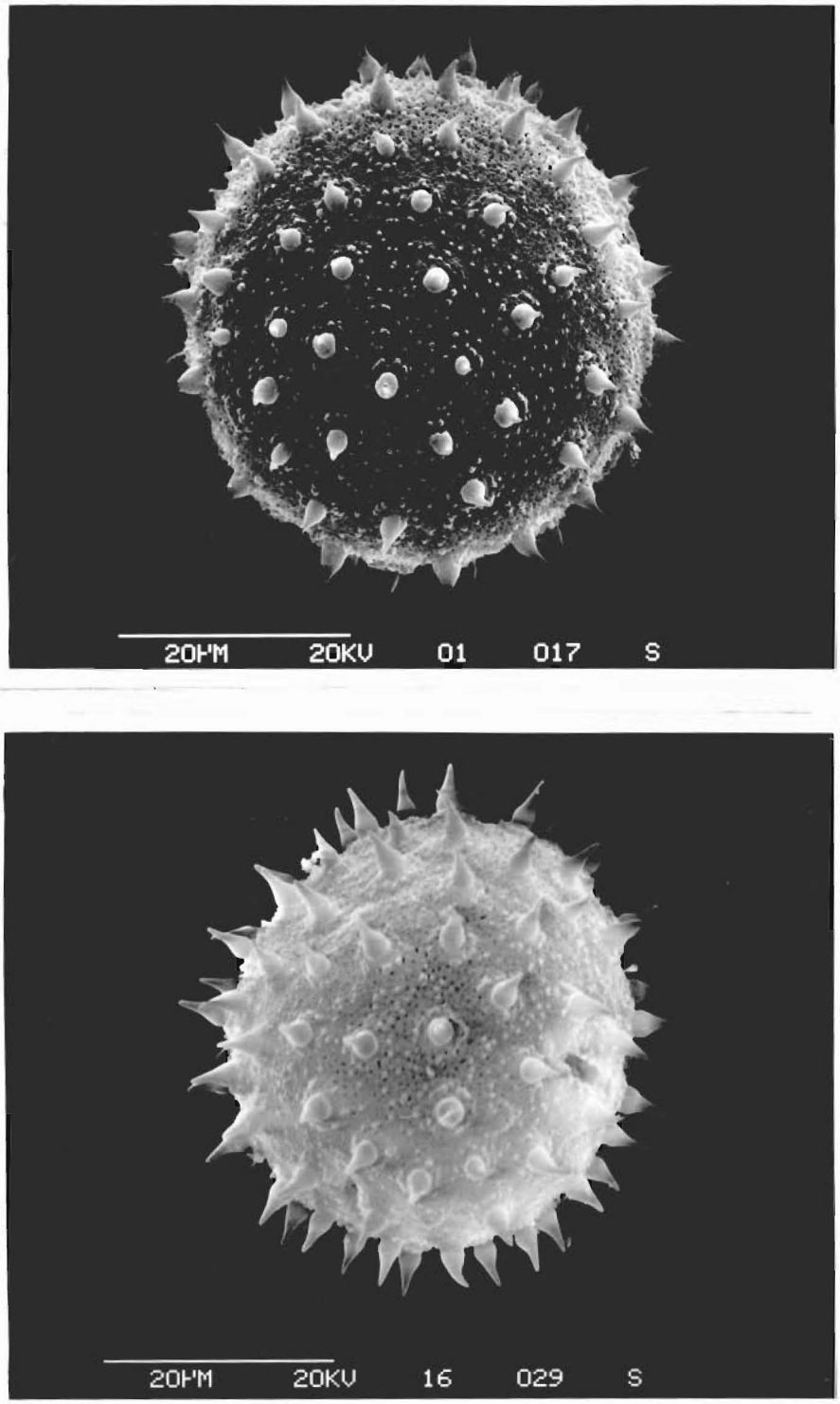



Figure 16 : Scanning electron micrographs of acetolysed pollen grains of *H. sexstylosa* var. *ovata*.

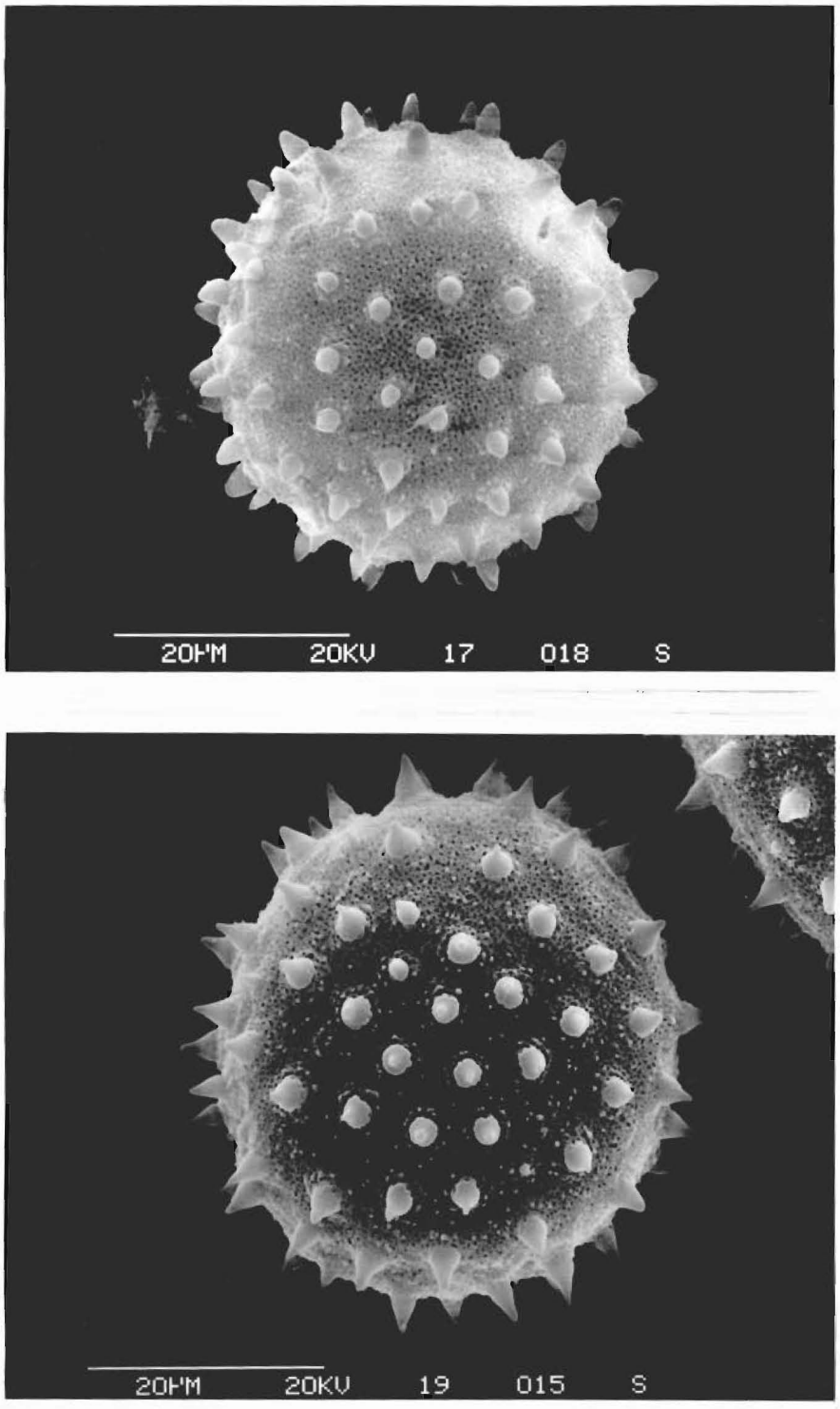
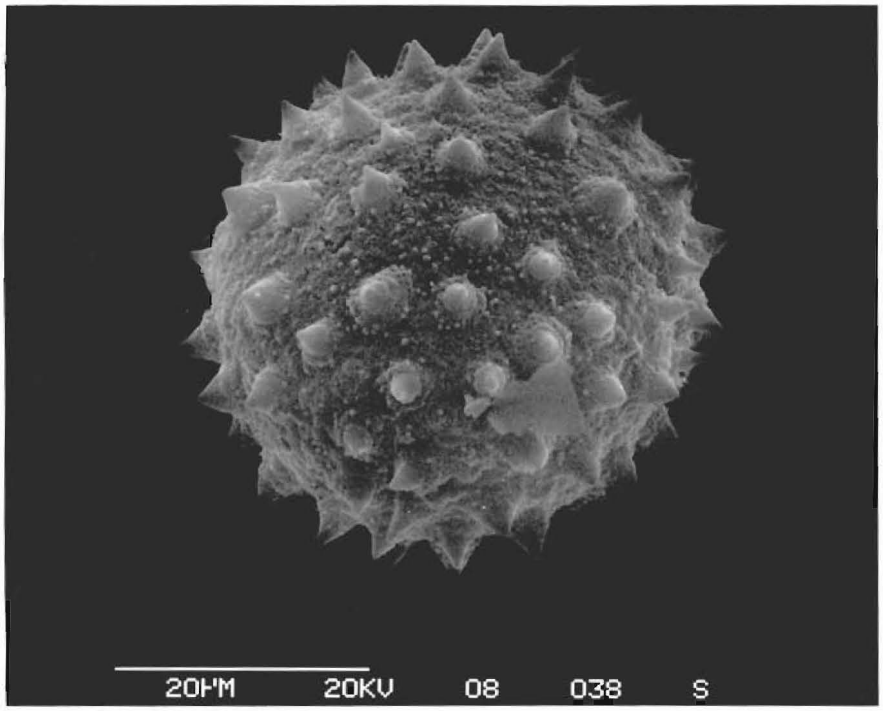



Figure 17 : Scanning electron micrographs of acetolysed pollen grains of *H. populnea*.



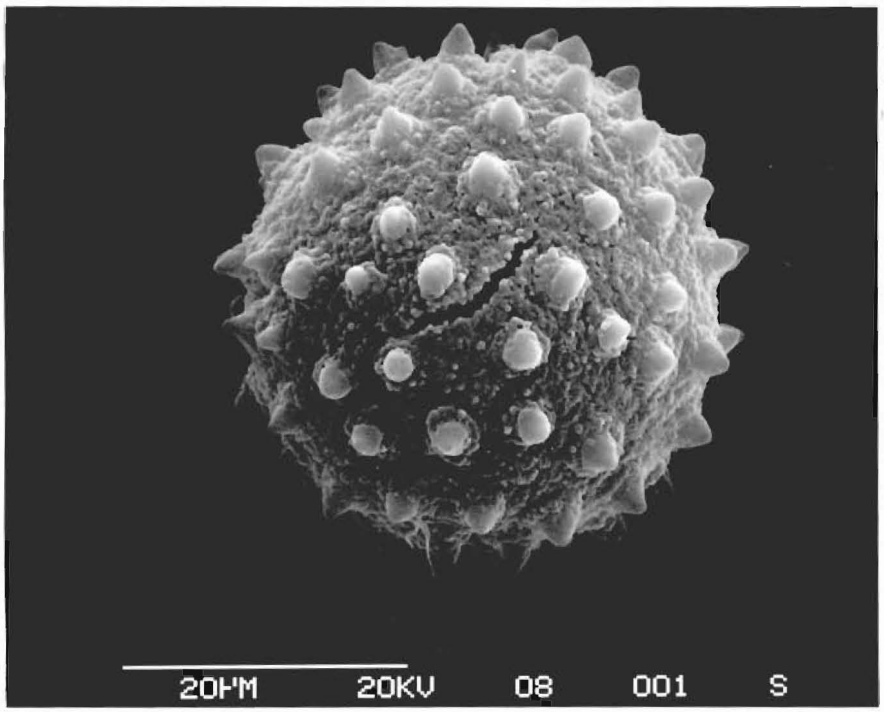




Figure 18 : Scanning electron micrographs of acetolysed pollen grains of *H. populnea* "Poor Knights".

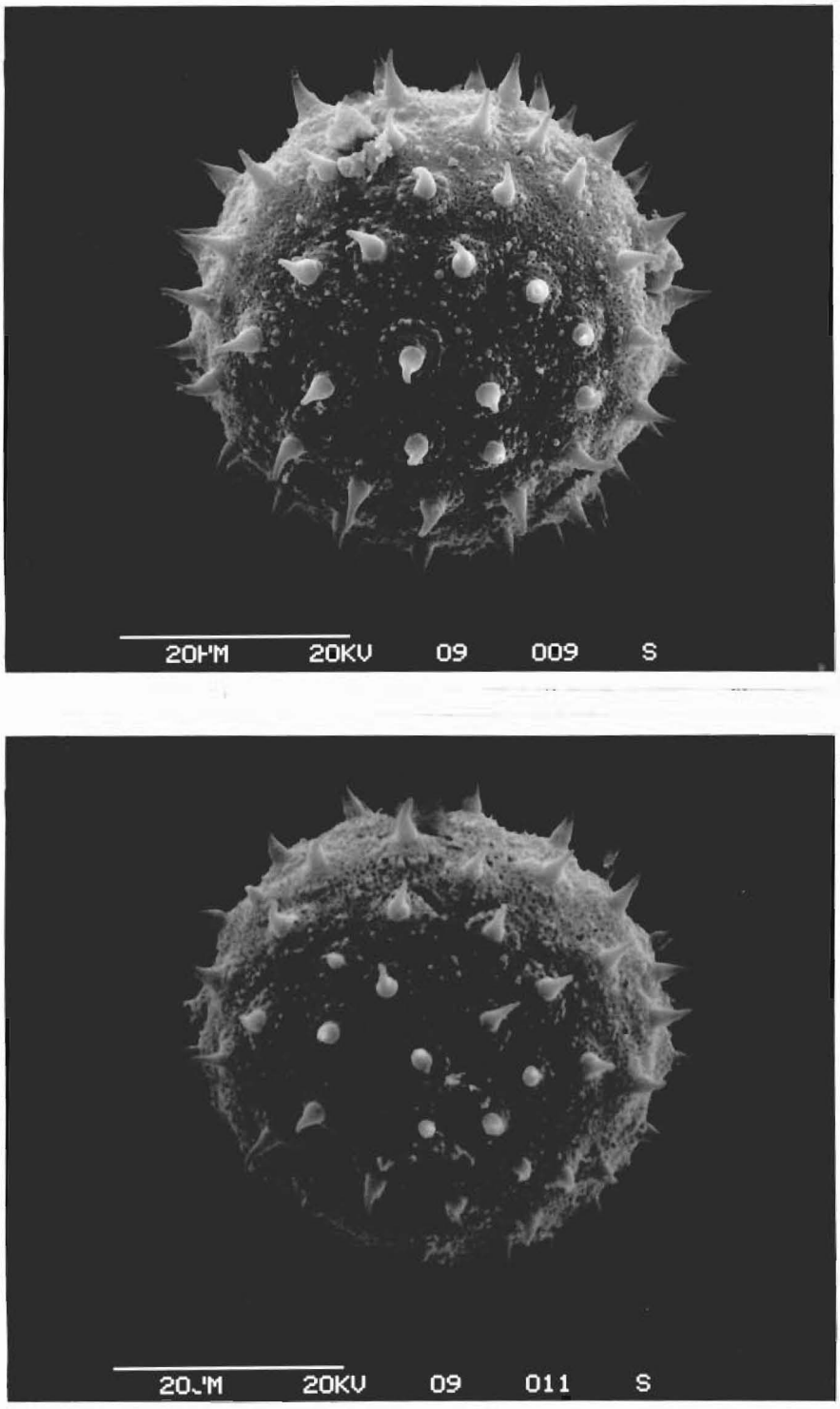



Figure 19 : Scanning electron micrographs of acetolysed pollen grains of *H*. "tararua".

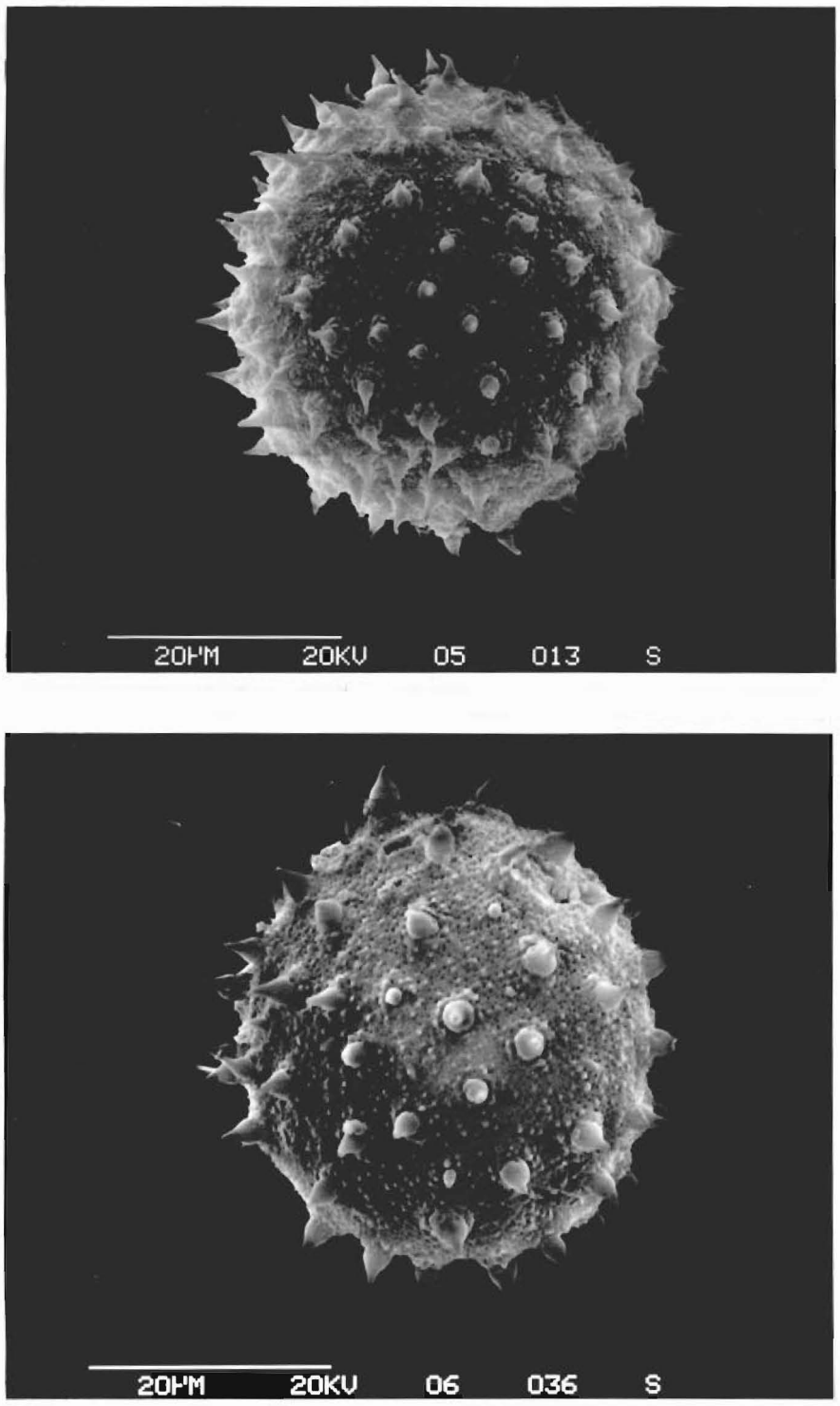



Figure 20 : Scanning electron micrographs of acetolysed pollen grains of *H. angustifolia*.

removed from the voucher specimens collected for the initial investigation of these two taxa. Methods of pollen collection and preparation for these taxa were the same as for other herbarium material.

Each sample consisted of six grains from a single whole flower, as individual anthers could not be adequately distinguished. The flowers which gave the greatest yields of pollen were newly opened as noted in Section 2.2.1.

## 2.3 METHODS OF POLLEN PREPARATION

### 2.3.1 Preparation of Fresh Pollen

Each sample of fresh pollen was transferred directly from the storage bags to numbered SEM mounts. Each mount was covered with a small square of double-sided sellotape over which the individual anther was shaken or lightly brushed. The mounts were then transferred to the Edwards 306 Vacuum Coater where they were coated with 50 nm of carbon and 50 nm of gold-palladium.

### 2.3.2 Preparation of Herbarium Material

#### 2.3.2.1 Preparation for the Light Microscope

Individual flowers were removed from herbarium sheets for the preliminary analysis of the *Hoheria* genus (Section 2.2.2.1). The following method used to acetolyse the pollen is based on Erdtman's acetolysis method (Erdtman, 1960).

(1) Individual flowers were placed directly into numbered centrifuge tubes.

(2) Approximately 5.0 ml of 10% potassium hydroxide was added to each tube to separate the pollen from the other flower parts. The tubes were left for 15 minutes and then heated in a 60°C water bath for ten minutes. During this time the contents were agitated with a glass stirring rod to assist separation of pollen from other flower parts.

(3) The contents were then flushed through a 500 μm sieve into a small beaker using distilled water. This liquid was then centrifuged and decanted off, leaving behind a pollen palette.

(4) Equal parts (2.0-3.0 ml) of glacial acetic acid were added to each tube, which were then centrifuged and decanted again.

(5) Equal parts of Erdtman's acetolysis mixture (one part concentrated sulphuric acid to nine parts acetic anhydride) were added to the centrifuge tubes. The tubes were then heated in a boiling water bath for four minutes and stirred continuously. They were then centrifuged and decanted again.

(6) Equal parts of glacial acetic acid were added to the tubes which were stirred, centrifuged and decanted.

(7) Finally distilled water was added to each tube and they were again centrifuged and decanted. This step was repeated.

After the final step the pollen was transferred to reference slides for viewing under the light microscope. A pasteur pipette was used to draw in the pollen at the bottom of the centrifuge tube and transfer it to a microscope slide positioned on a hot plate. A few drops

of glycerine jelly, previously heated to a liquid in a hot water bath, were then added to the pollen and stirred in with a toothpick. Staining was unnecessary as the pollen grains were coloured light orange by the acetolysis process. A cover slip was placed over the mixture which was then left to cool.

2.3.2.2 Preparation for the SEM

For the *H. populnea* et al. study flowers were removed from herbarium sheets and acetolysed in the same way as those for the light microscope study. Following acetolysis samples were put through a dehydration series, filtered and critical point dried in preparation for observation under the SEM.

The dehydration series consisted of increasing concentrations of ethanol in which the pollen was left for approximately 45 minutes. After this time the mixture was centrifuged and decanted and the next concentration of ethanol added. The concentrations were 20%, 50%, 70%, 95%, 100% (twice), followed by a mix of 50% pure ethanol and 50% pure amyl acetate, and finally a 100% mixture of amyl acetate. At this stage the centrifuge tubes were corked and left overnight. Amyl acetate probably removes residual oils or waxes remaining after acetolysis and dehydration (Pocknall 1979), and is miscible with the carbon dioxide used in critical point drying.

The following morning the samples were filtered through Whatman number 1 filter paper to drain off the excess amyl acetate. The filter paper containing the pollen was then folded over and placed into small vials

inside the drying chamber for critical point drying (Anderson 1951; Boyde and Wood 1969). The amyl acetate is replaced by liquid carbon dioxide ~~injected into the chamber~~ which, on forming a gas, dries the specimens. During this process carbon dioxide passes from a liquid to a gas without altering the structure of the pollen grains. The desiccated pollen, still on the filter paper, is then ready for mounting. However, double-sided sellotape was not used, as the fresh *H. lyallii-H. glabrata* pollen previously prepared for the SEM had been found to sink into the sellotape after about four weeks. As a substitute a strip of double-sided sellotape (approximately 1.5 cm long) was left in 10 ml of chloroform for 15 minutes. During this time the sellotape adhesive was dissolved off its backing and into the chloroform making a glue (Vaughan Meyers, Botany Division, D.S.I.R.). A drop of the glue was then spread uniformly over each SEM mount and left for approximately 30 seconds to dry. Under a binocular microscope the filter paper was unfolded and the mounts gently pressed onto the concentration of pollen grains. The mounts were then coated in an E5000 Diode Sputtering System at 20 kV for five minutes. This deposited sixty nm of gold over their surfaces. All pollen for the *H. populnea* et al. study was sputter-coated, in favour of using the Edwards 306 Coater. This was because the Edwards deposited thick coats of carbon and gold-palladium partially concealing the surface ornamentation of the pollen grains. The pollen grains were then ready for loading into the SEM.

### 2.3.3 Scanning Electron Microscopy

With the exception of the light microscope study all pollen grains were studied and photographed on a Cambridge 250 Mark II Scanning Electron Microscope. With the machine operating at 20 kV, all grains were magnified approximately 1500x. (While the display read 1500x, manual calculations using the bar scale on each photograph showed magnifications to vary between 1300x and 1700x. This variation was allowed for in all grain and spine measurements). All micrographs were taken using a Nikon EM 35 mm camera loaded with Ilford FP4 film.

### 2.3.4 Measurements

The micrographs were approximately 11 cm x 8 cm and developed onto Ilfobrom 1.1 P glossy paper, dried and glazed. Spine characters were measured on the micrographs under a Kyowa SDZ-AL binocular microscope, using an ocular scale. These measurements were converted to micrometers using the bar scale on each photograph. Grain characters for each micrograph were measured using a millimeter rule and also converted to micrometers. Measurements were made to two significant figures.

## 2.4 STATISTICAL ANALYSIS : INVESTIGATION DESIGNS

### 2.4.1 Introduction

The purpose of the initial investigation of *H. glabrata* and *H. lyallii* was to quantify the pollen variation at different levels within these two taxa.

Therefore a nested analysis of variance was used on the data. For the major *H. glabrata-H. lyallii* analysis and the *H. populnea* et al. study discriminant analysis was used to determine whether pollen characters could be used to reliably separate the different taxa. The Statistical Package of the Social Sciences (SPSSx) was used for these analyses, on the Prime Computer at the University of Canterbury.

### 2.4.2 Initial Investigation of *H. glabrata* and *H. lyallii*

#### 2.4.2.1 Introduction

The initial investigation was set up to:

(1) determine the future sampling strategy for these two taxa by quantifying variation in pollen at different levels within a tree; and

(2) uncover and resolve any sampling or statistical difficulties encountered in this study, in preparation for the major *H. glabrata-H.lyallii* analysis.

#### 2.4.2.2 Analysis of Variance

The aim of the analysis of variance (ANOVA) was to discover the amount of variation at different levels within and between the sampled trees. Therefore the ANOVA consisted of six nested levels: spines within grains, grains within anthers, anthers within flowers, flowers within branches, branches within trees and trees within taxa. This number of levels, combined with uneven replicates at the grain level (Section 2.2.1.2), necessitated the construction of a complex command file to

run this program in SPSSx. A separate ANOVA was computed for each of the nine characters to determine the level at which significant variation was occurring for the individual character (Section 3.1). These results were supported by percentage variation calculations determined for each character at every level (Section 3.1, Table 3) using the mean squares listed in the ANOVA tables . The results determined the sampling strategy for the subsequent analysis.

To ascertain the number of measurements to make at each level an 'optimal allocation of resources' was calculated (Sokal and Rohlf, 1981) with help from Dr. D. Kelly (Section 3.1). Using mean squares and percentage of variation, the optimum number of replicates to measure for each character at every level was determined. This reduced and streamlined the major *H. glabrata-H. lyallii* analysis where samples were removed from only those levels at which significant variation occurred.

2.4.2.3 Discriminant Analysis

A discriminant analysis was carried out in an attempt to separate the two taxa using their pollen characters. The analysis used all entered data to undertake a stepwise variable selection. This revealed the descending order of importance of the nine pollen characters on their ability to discriminate between the two taxa, and calculated a discriminant function to describe this. The computer selected the data, one spine at a time, and used the discriminant function to allocate to each spine a discriminant score. This score was then used to classify

each spine into one of the two groups, (or in this case taxa). The computer will classify the data into as many groups as are stated in the command file. Groups may correspond to any defineable cluster within the data, such as those grains clustered by locality, or those clustered within a tree, as in Sections 2.4.3 and 2.4.4.

### 2.4.3 Major *H. glabrata-H. lyallii* Analysis

The purpose of this investigation was to attempt to separate the sampled trees into two groups, corresponding to taxa, on the basis of pollen characters using a discriminant analysis. To do this 24 trees, 12 of each taxon, were sampled using the following reduced sampling strategy (Section 2.2.1.3): three spines per grain from six grains per anther, removed from two anthers per tree for both taxa (Figure 14). The computer therefore analysed 36 'cases' (spines) per tree. The four trees sampled for the initial investigation were included in both this analysis and the *H. populnea* et al. study.

All pollen data underwent two discriminant analyses. The first analysis classified the pollen of the 24 trees into two groups. The computer was given only two options to choose from, *H. glabrata* and *H. lyallii*. In the second analysis the program command file was altered to classify the pollen into 24 groups, corresponding to the individual trees. This was to discover any intra-taxon groupings present, such as may be caused by climatic or locality factors.

### 2.4.4 *H. populnea* et al. Study

The *H. populnea* et al. study was, due to time constraints, only a brief glimpse at the pollen variation present in the remaining taxa of this genus. Its purpose was to ascertain whether the pollen of this genus would fall into distinct groups, or whether a degree of overlap occurred in pollen morphology. Herbarium sheets were the sole source of pollen samples for this analysis, consequently intensive 'within tree' sampling was not possible. With the exception of *H. sexstylosa* and *H. sexstylosa* var. *ovata* only two flowers per species were sampled (Section 2.2.2.3).

In the first analysis all data were analysed for classification into eight groups, corresponding to the eight taxa present. For the second analysis all data were used for classification into 21 groups, corresponding to the 21 trees in the analysis. The purpose of this was twofold:

(1) to determine the amount of pollen variation in individual trees from each taxon, and

(2) to disclose any groupings between trees of different taxa.

In the third analysis the data collected from *H. sexstylosa* and *H. sexstylosa* var. *ovata* were analysed for classification into two groups. Four and five trees respectively were sampled from these taxa (Section 2.2.2.3). In the fourth analysis the data of *H. sexstylosa* and *H. sexstylosa* var. *ovata* were also analysed for classification into two groups. However only two trees of each taxon were sampled.

The third and fourth discriminant analyses were undertaken to discover the outcome of the groupings given more data per taxon, but less options of classification. It was possible that the degree of correct classification was inversely related to the number of groups the computer could select to classify spines into, and the quantity of data available in each group.

# CHAPTER 3

# RESULTS

## 3.1 INITIAL INVESTIGATION OF *H. GLABRATA* AND *H. LYALLII*

The analysis of variance for the initial investigation revealed that using the nine characters highly significant intraspecific variation in pollen morphology occurred at the grain, anther and tree levels for each of the two taxa (Table 3). Significant variation was not present at either the branch or flower level. Consequently sampling concentrated on the areas of greatest variation; the grains, anthers and trees from the two taxa.

To determine the number of replicates to measure at each level an optimal allocation of resources was calculated (Section 2.4.2.2). The character 'spine width' is given as an example of this method (Table 4). The amount of variation at each level was consistent throughout all characters. Consequently for the major *H. glabrata-H. lyallii* analysis three spines were sampled per grain, six grains per anther and two anthers per tree. The number of grains sampled per anther was increased from the two shown as minimum by the optimal allocation of resources (Table 4) to six, as this was the most variable level and there was enough time to complete the extra recording.

Table 3 : Percentage variation for each pollen character at each sampled level.

| Level | Spine Length | Spine Width | Mid-spine Width | Taper (%) | Taper Rate | Min'm Grain Diameter | Max'm Grain Diameter | Grain Ellipse | Spine Density |
|---|---|---|---|---|---|---|---|---|---|
| Taxon | 25.07 | 59.92 | 56.26 | 12.6 | 0.25 | 7.11 | 4.55 | 0.56 | 5.36 |
| Tree | 8.54 | 10.47 | 13.3 | 11.91 | 7.96 | 0.72 | 3.5 | 0.16 | 7.21 |
| Branch | 3.19 | 0.12 | .01 | 0.09 | 1.37 | 1.16 | 0.36 | 0.9 | 0.21 |
| Flower | 0.25 | 2.35 | 1.17 | 18.56 | 4.73 | 1.12 | 1.3 | 1.32 | 2.42 |
| Anther | 15.42 | 2.1 | 1.41 | 40.02 | 8.21 | 2.14 | 0.2 | 32.9 | 4.83 |
| Grain | 13.24 | 3.18 | 2.97 | 0.24* | 4.73* | 87.76* | 90.08* | 64.16* | 79.96* |
| Spine | 34.29* | 21.85* | 24.87* | 16.6* | 72.64* | | | | |

123 - Highly significant
123 - Significant
* - Computer program unable to test these figures for significance

**Table 4 : Optimal allocation of resources determined at each sampled level for spine width (Sokal and Rohlf, 1981)**

| Subgroups | True Value (n) | Value Used in Analysis |
|---|---|---|
| Number of spines/grain | 3.39 | 3 |
| Number of grains/anther | 1.93 | 2 |
| Number of anthers/flower | 0.94 | 1 |
| Number of flowers/branch | 0 | 0 |
| Number of branches/tree | 0 | 0 |
| Number of flowers/tree | 0.46 | 1 |

The above values were calculated using the formula

$$n = \sqrt{\frac{C_{GCA} S^2_{SCG}}{C_{SCG} S^2_{GCA}}}$$

n = optimal number of replicates per subgroup
C = cost
$S^2$ = variance
S = spines
G = grains
A = anthers
C = within

No significant difference was found to exist between character means, and therefore a discriminant analysis was run on the data in an attempt to separate the two taxa (Section 2.3.2.3). While nine pollen characters were measured or computed for the grains, the computer program selected only seven for the analysis.

The characters 'maximum grain diameter' and 'grain ellipse' were omitted as they did not aid in the discrimination of the two taxa. Maximum grain diameter was highly correlated with minimum grain diameter and therefore only the latter character was used. For grain ellipse there was no significant difference between the two

taxa. Consequently the inclusion of this character was unnecessary. As a result of poor univariate discrimination all seven characters were required to attempt a separation of these two taxa.

The results of the discriminant analysis showed that pollen variation in the four trees sampled was itself variable (Table 5). The computer treated the three spines measured on each grain as separate cases. Each 'spine-case' was based on the characteristics of that spine as well as the characteristics of the grain from which it was taken. For *H. glabrata* 47% and 0% of the spines on each tree were misclassified into *H. lyallii*. In contrast *H. lyallii* recorded misclassifications of 7% and 14% per tree into *H. glabrata*. Individual branches within trees also exhibited considerable variation, with differences of up to three times the number of cases misclassified on one branch as compared to another.

Table 5 : Percentage of misclassified spines within branches for the initial investigation of *H. glabrata* and *H. lyallii*

| Taxon | Tree Number | Branch Number | Percentage Misclassified | Mean Percentage Misclassified |
|---|---|---|---|---|
| *H. glabrata* | 1 | 1 | 50 | |
| | | 2 | 75 | |
| | | 3 | 17 | 47 |
| | 2 | 1 | 0 | |
| | | 2 | 0 | |
| | | 3 | 0 | 0 |
| *H. lyallii* | 1 | 1 | 8 | |
| | | 2 | 6 | 7 |
| | 2 | 1 | 6 | |
| | | 2 | 22 | 14 |

In total 76% of *H. glabrata* spines and 90% of *H. lyallii* spines were correctly classified (Table 6). These results are summarised in Figure 21, which graphs the intergradation of the pollen. This graph clearly shows that two trees, one from each taxon, are well separated on their pollen characteristics. The remaining two trees intergrade, particularly the extremely misclassified tree of *H. glabrata*.

Table 6 : Predicted group membership for the initial investigation of *H. glabrata* and *H. lyallii*

| Actual Group | Predicted Group | |
|---|---|---|
| | *H. glabrata* | *H. lyallii* |
| *H. glabrata* | 76.4% | 23.6% |
| *H. lyallii* | 10.4% | 89.6% |

Total percent of spines correctly classified = 85.19%

## 3.2 MAJOR *H. GLABRATA*-*H. LYALLII* ANALYSIS

This investigation set out to see how many of the sampled trees of *H. glabrata* and *H. lyallii* could be separated using discriminant analysis, based on pollen characteristics. As with the initial investigation, the 24 trees could not be adequately separated on these grounds (Figure 22). With the choice of two taxonomic groups to classify the 936 spines into, only 677 (72%) were correctly classified (Table 7). Variation was again widespread, with the computer frequently classifying different spines

Figure 21. Frequency of Discriminant Scores for the Initial Investigation of H. glabrata and H. lyallii.

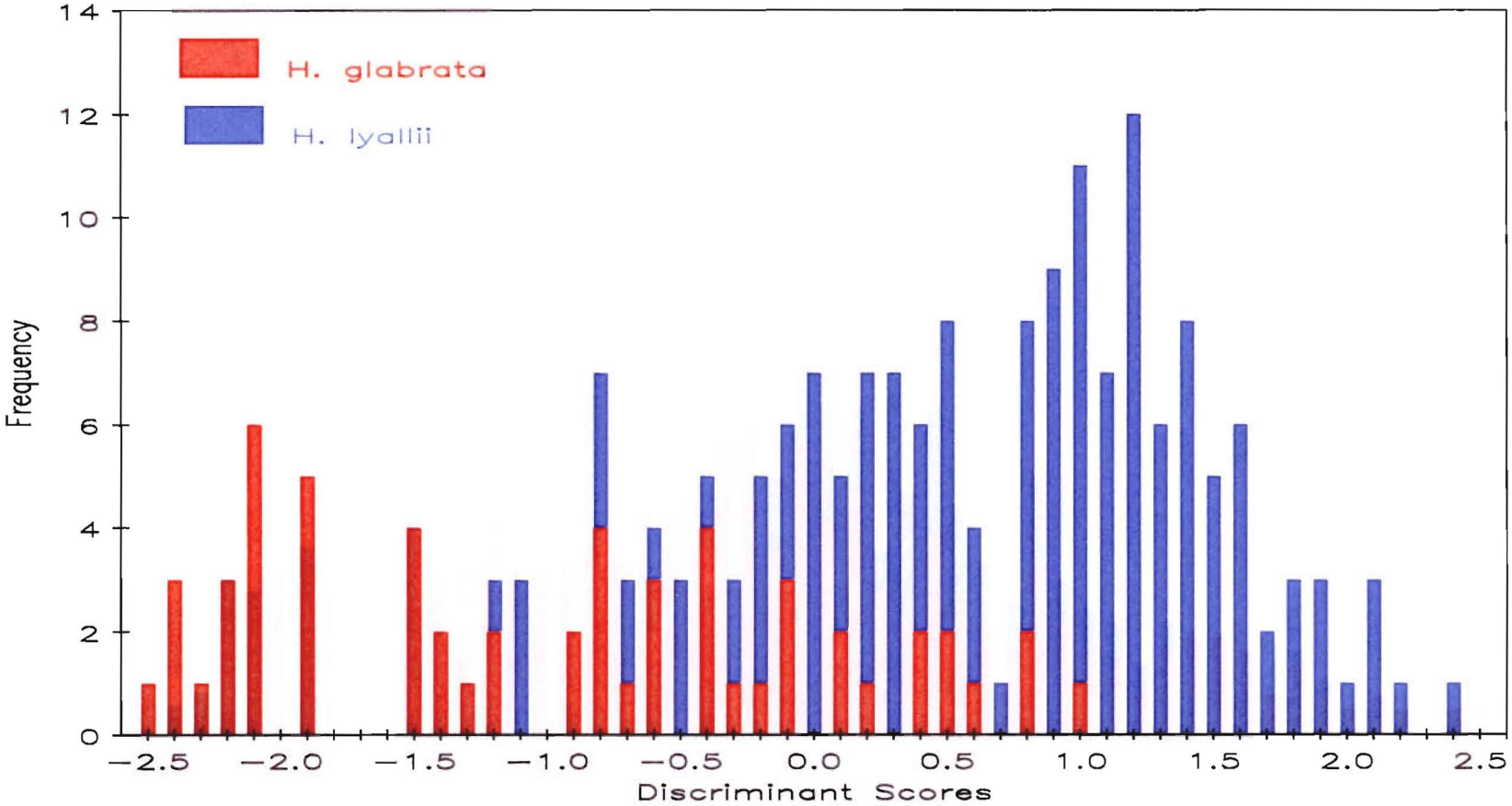
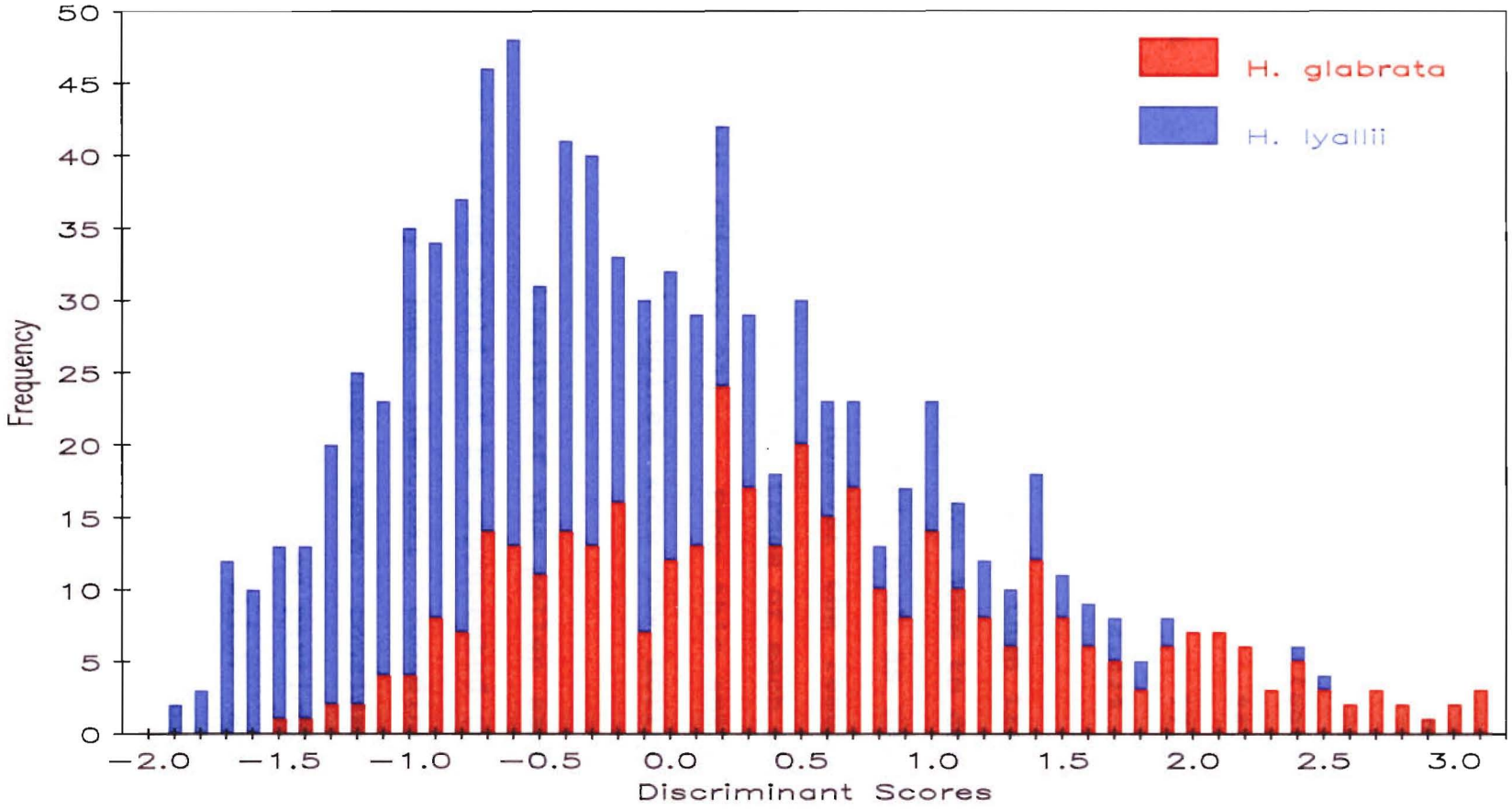


Figure 22. Frequency of Discriminant Scores for the Major Analysis of H. glabrata and H. lyallii.



on the same grain into either taxa. Clearly for this to occur spine variation was extensive.

**Table 7 : Predicted group membership for the major *H. glabrata*-*H. lyallii* analysis**

| Actual Group | Predicted Group | |
|---|---|---|
| | *H. glabrata* | *H. lyallii* |
| *H. glabrata* | 65.9% | 34.1% |
| *H. lyallii* | 23.0% | 77.0% |

Total percent of spines correctly classified = 72.33%

In a second analysis the 24 trees were then treated as 24 groups, rather than as two. This was done to detect whether any intra-taxon groupings existed, such as may be caused by environmental factors. However the resulting scattergram showed that pollen from the trees of each taxon was largely intermixed (Figure 23). From this analysis only 382 (41%) of the total spines were now correctly classified.

Due to the extreme similarity of the pollen sampled from each tree, many spines could not be represented on the scattergram because they had identical coordinates. Consequently a number of trees were considerably under-represented on the graph. This applies particularly to those trees with 'normal' pollen, which appear intermixed with other trees in the central area of the graph. In contrast those trees which have slightly different pollen are well defined and therefore have a large number of correctly classified spines, with many of their points represented on the scattergram. Such an

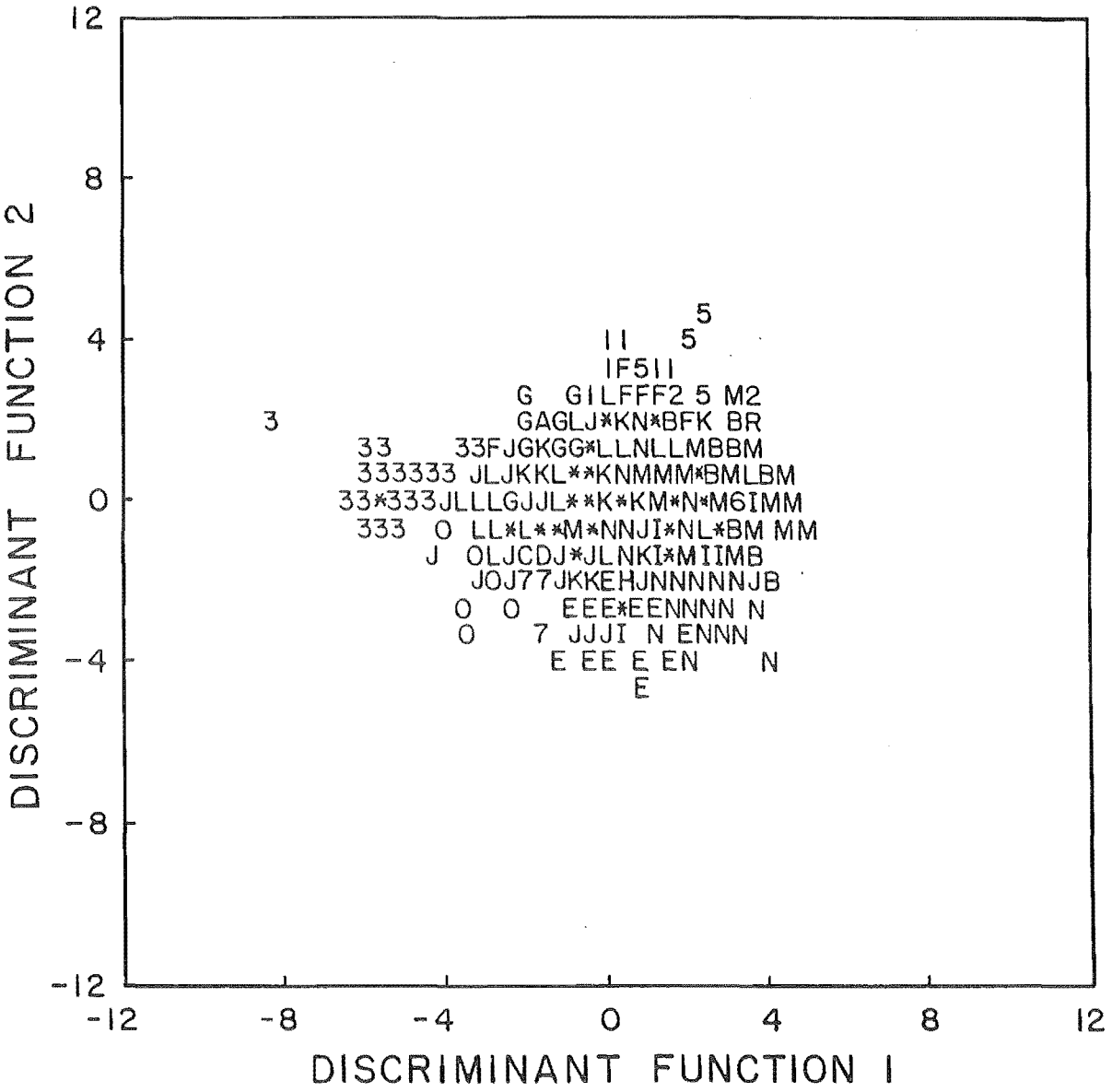



Figure 23 : Results of discriminant classification for the major *H. glabrata-H. lyallii* analysis, with the 24 trees grouped individually.
(Legend on following page).

Legend for Figure 23

| Symbol (Tree No.) | Taxon |
|---|---|
| 1 | *H. lyallii* |
| 2 | *H. lyallii* |
| 3 | *H. lyallii* |
| 4 | *H. lyallii* |
| 5 | *H. lyallii* |
| 6 | *H. lyallii* |
| 7 | *H. lyallii* |
| A | *H. lyallii* |
| B | *H. lyallii* |
| C | *H. lyallii* |
| F | *H. lyallii* |
| G | *H. lyallii* |
| 8 | *H. glabrata* |
| 9 | *H. glabrata* |
| O | *H. glabrata* |
| D | *H. glabrata* |
| E | *H. glabrata* |
| H | *H. glabrata* |
| I | *H. glabrata* |
| J | *H. glabrata* |
| K | *H. glabrata* |
| L | *H. glabrata* |
| M | *H. glabrata* |
| N | *H. glabrata* |
| * | Group centroids |

example is tree number three which has 94% of its spines correctly classified by the analysis. This situation also occurs on the scattergrams of the *H. populnea* et al. study.

The variable nature of these results is illustrated more clearly in Table 8. The percentage of correctly classified spines per tree ranged from 94% down to 6%. The higher figure however is rather irregular, as when viewed under the SEM all grains from this tree of *H. lyallii* had both a low spine density and extremely short spines (Figure 24). This tree was one of five sampled from Pylon Gully, the remaining four of which had 'typical' pollen grains. While the gross morphology of this tree appeared normal for *H. lyallii*, the pollen grains suggest that it may be genetically distinct. Table 8 shows the great variability which exists in pollen from different trees. While some trees have extremely variable pollen, others have very similar pollen.

The data from the four trees of the initial investigation, included in this analysis, were then studied further (Table 9). When included in a larger analysis and separated as individual trees, their spines were no longer classified into the other tree of the same taxon, or into the remaining two trees of the initial investigation. The majority of these misclassified spines were grouped in with the other trees of the major analysis.

Table 8 : Predicted group membership of spines for individual trees of the major *H. glabrata*–*H. lyallii* analysis.

| Taxon | Tree Number | Scattergram Symbol | Percent Correctly Classified | Percent Misclassified *H. glabrata* | Percent Misclassified *H. lyallii* |
|---|---|---|---|---|---|
| *H. glabrata* | 1 | 8 | 50.0 | 19.4 | 30.6 |
| | 2 | 9 | 63.9 | 16.7 | 19.4 |
| | 3 | 0 | 69.4 | 22.3 | 8.4 |
| | 4 | D | 19.4 | 41.8 | 38.8 |
| | 5 | E | 52.8 | 25.1 | 22.2 |
| | 6 | H | 22.2 | 36.1 | 41.6 |
| | 7 | I | 16.7 | 61.2 | 22.1 |
| | 8 | J | 27.8 | 39.0 | 33.3 |
| | 9 | K | 27.8 | 41.8 | 30.4 |
| | 10 | L | 5.6 | 58.4 | 36.1 |
| | 11 | M | 50.0 | 27.8 | 22.3 |
| | 12 | N | 50.0 | 27.8 | 22.3 |
| *H. lyallii* | 1 | 1 | 69.4 | 0.0 | 30.6 |
| | 2 | 2 | 47.2 | 41.7 | 11.2 |
| | 3 | 3 | 94.4 | 2.8 | 2.8 |
| | 4 | 4 | 13.9 | 52.7 | 33.4 |
| | 5 | 5 | 36.1 | 2.8 | 61.1 |
| | 6 | 6 | 25.0 | 27.8 | 47.2 |
| | 7 | 7 | 44.4 | 47.3 | 8.4 |
| | 8 | A | 36.1 | 33.4 | 30.6 |
| | 9 | B | 61.1 | 8.4 | 30.6 |
| | 10 | C | 63.9 | 25.0 | 11.2 |
| | 11 | F | 22.2 | 26.6 | 51.2 |
| | 12 | G | 34.7 | 38.8 | 26.5 |

Total percent of spines correctly classified = 40.81%

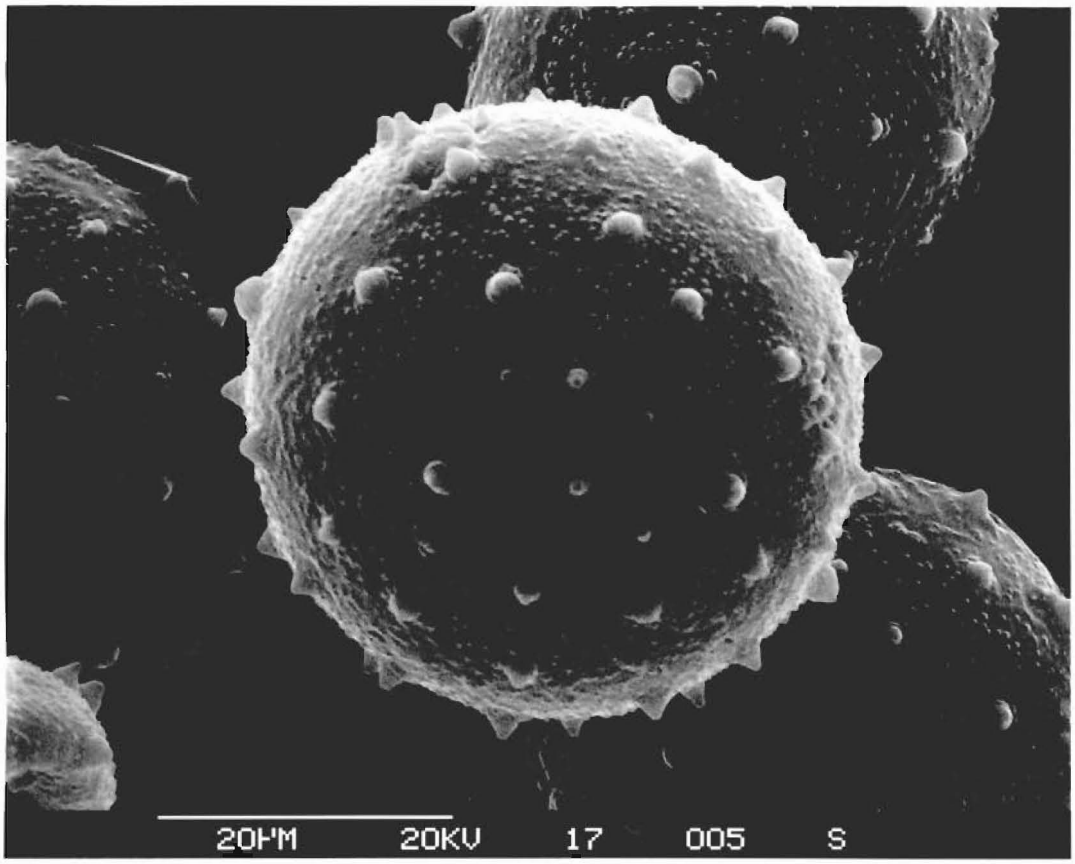



Figure 24 : Unusual pollen grain taken from a tree of *H. lyallii*, Pylon Gully, near Cass. This morphological type was found to be unique to this tree.

Table 9 : Predicted group membership of the four trees of the initial investigation, when grouped with all trees of the major *H. glabrata-H. lyallii* analysis

| Actual Group | Predicted Group (%) | | | | Percentage Misclassified (%) | |
|---|---|---|---|---|---|---|
| | G1 | G2 | L3 | L4 | *H. glabrata* | *H. lyallii* |
| G1 | 19.4 | 0 | 2.8 | 8.3 | 41.8 | 27.7 |
| G2 | 0 | 52.8 | 0 | 0 | 21.5 | 22.2 |
| L3 | 4.2 | 0 | 22.2 | 13.9 | 22.4 | 37.3 |
| L4 | 13.9 | 0 | 5.6 | 34.7 | 24.9 | 20.9 |

G1,G2 = *H. glabrata*
L3,L4 = *H. lyallii*

## 3.3 *H. POPULNEA* ET AL. STUDY

### 3.3.1 Introduction

Four discriminant analyses were undertaken (Section 2.4.4) on the data collected from the eight taxa of *Hoheria*. The analyses were computed to

(1) compare with the results of the major *H. glabrata* - *H. lyallii* analysis, and

(2) to offer an insight into the nature of pollen variation in the remaining taxa of this genus.

### 3.3.2 Discriminant Analysis By Taxa

The first discriminant analysis classified the 21 trees into eight groups, corresponding to taxa (Figure 25). As seen in the scattergram none of the eight taxa were adequately separated from each other. Notably *H. sexstylosa* and *H. sexstylosa* var. *ovata*, which are

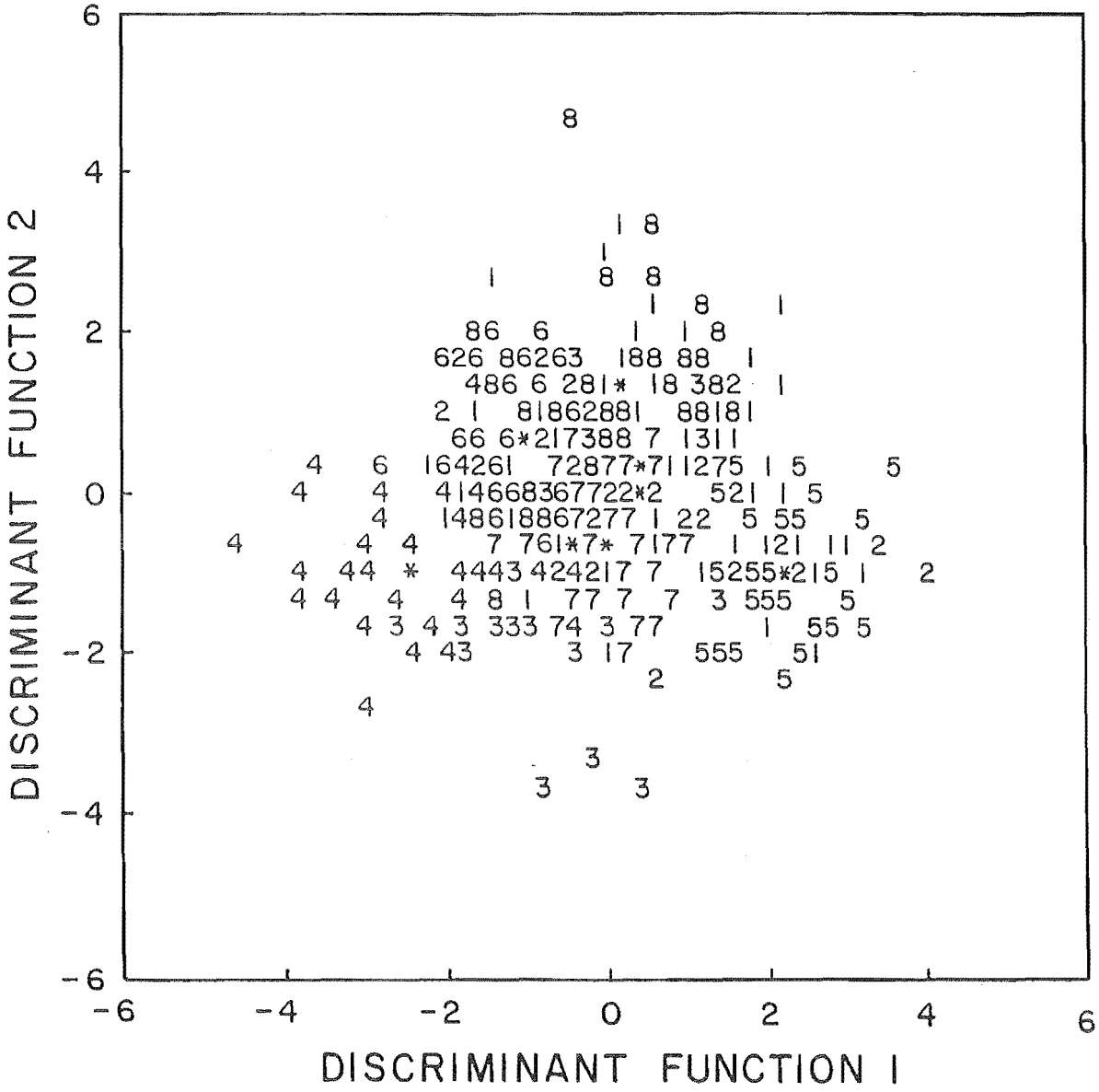



Figure 25 : Results of discriminant classification for the *H. populnea* et al. analysis, with the 21 trees grouped into eight taxa.
(Legend on following page).

Legend for Figure 25

| Symbol | Taxon |
|---|---|
| 1 | *H. sexstylosa* var. *ovata* |
| 2 | *H. sexstylosa* |
| 3 | *H. angustifolia* |
| 4 | *H. populnea* "Poor Knights" |
| 5 | *H.* "tararua" |
| 6 | *H. populnea* |
| 7 | *H. glabrata* |
| 8 | *H. lyallii* |
| * | Group centroids |

represented by the greatest number of trees per taxon in this analysis, have points disseminated throughout the scattergram. However both *H.* "tararua" and *H. populnea* "Poor Knights" form reasonably defineable clusters.

These results reflect the number of correctly classified spines listed in Table 10. While more than 80% of spines for each *H.* "tararua" and *H. populnea* "Poor Knights" were correctly classified, less than 30% of *H. sexstylosa* and *H. sexstylosa* var. *ovata* were correctly classified.

Table 10 also reveals an important trend between *H. glabrata* and *H. lyallii*. For both taxa 75% of the spines were correctly classified. However the remaining 25% misclassified for each taxon did not group in with each other, as was expected in such apparently closely related taxa. Instead they tended to classify into the other six taxa.

### 3.3.3 Discriminant Analysis By Trees

The second discriminant analysis classified the 21 trees into 21 groups. The resulting scattergram (Figure 26) shows the trees to consist of scattered, intermixed points. While no specific groupings existed between trees of different taxa, the scattergram revealed the presence of intra-taxon patterns. *H. sexstylosa* for example is represented by two groups of trees. The first group, trees three and seven, consists of pollen very similar in morphology to the other taxa in the analysis. This is shown by the central representation on the scattergram of relatively few disseminated points. These trees had a

Table 10 : Predicted group membership of spines for all eight taxa of the *H. populnea* et al. study.

| Actual Taxon | Predicted Taxon (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *H. sexstylosa* var. *ovata* | *H. sexstylosa* | *H. angustifolia* | *H. populnea* "Poor Knights" | *H.* "tararua" | *H. populnea* | *H. glabrata* | *H. lyallii* |
| *H. sexstylosa* var. *ovata* | 24.4 | 12.2 | 10.0 | 6.7 | 12.2 | 6.7 | 8.9 | 18.9 |
| *H. sexstylosa* | 12.5 | 26.4 | 16.7 | 0.0 | 6.9 | 12.5 | 18.1 | 6.9 |
| *H. angustifolia* | 30.6 | 5.6 | 47.2 | 0.0 | 5.6 | 8.3 | 0.0 | 2.8 |
| *H. populnea* "Poor Knights" | 0.0 | 0.0 | 5.6 | 80.6 | 0.0 | 2.8 | 8.3 | 2.8 |
| *H.* "tararua" | 0.0 | 11.1 | 0.0 | 0.0 | 83.3 | 0.0 | 5.6 | 0.0 |
| *H. populnea* | 2.8 | 2.8 | 0.0 | 0.0 | 0.0 | 66.7 | 11.1 | 16.7 |
| *H. glabrata* | 0.0 | 5.6 | 11.1 | 0.0 | 0.0 | 5.6 | 75.0 | 2.8 |
| *H. lyallii* | 8.3 | 8.3 | 0.0 | 8.3 | 0.0 | 0.0 | 0.0 | 75.0 |

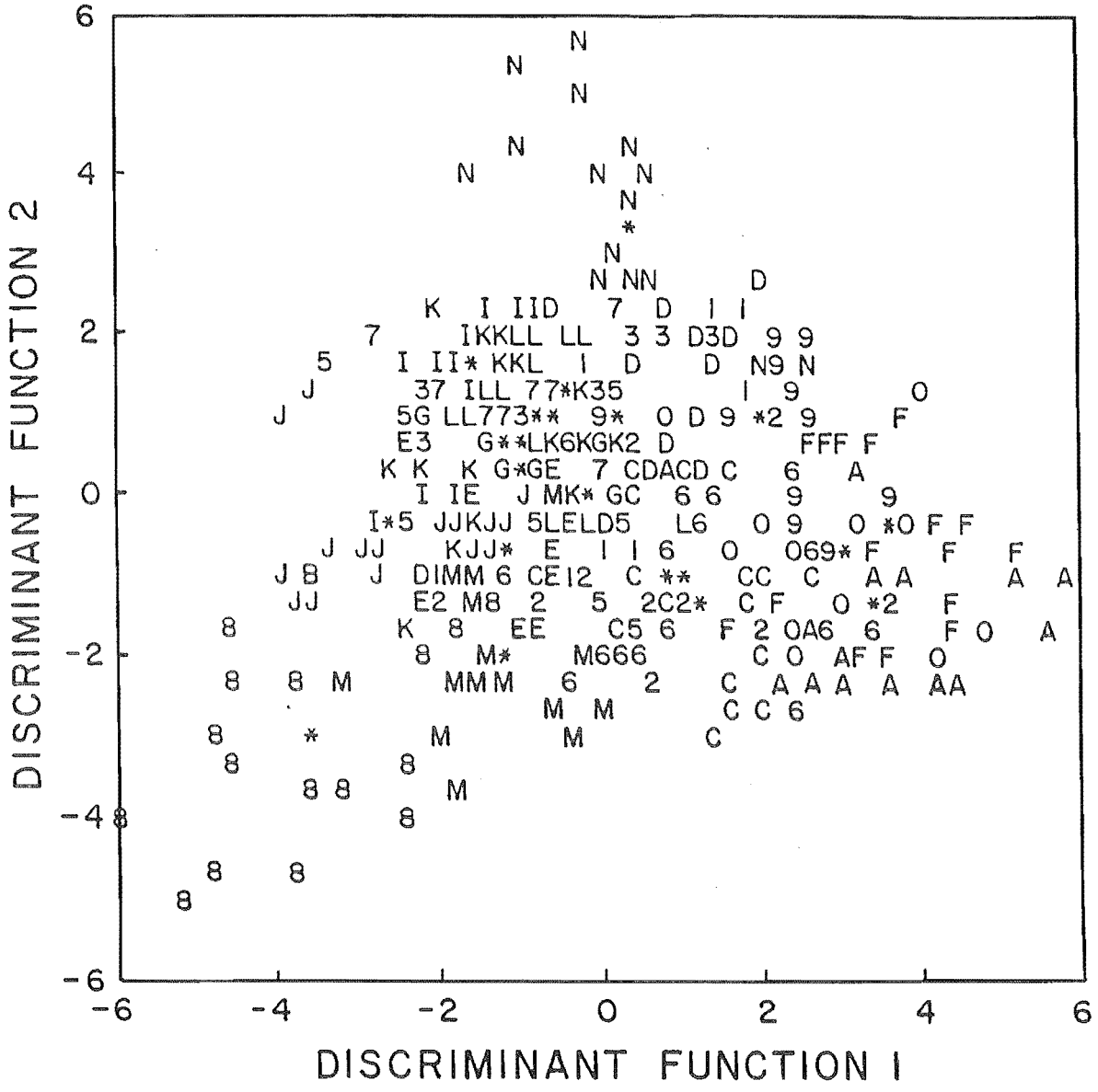



Figure 26 : Results of discriminant classification for the *H. populnea* et al. analysis, with the 21 trees grouped individually.
(Legend on following page).

Legend for Figure 26

| Symbol (Tree No.) | Taxon |
|---|---|
| 1 | *H. sexstylosa* var. *ovata* |
| 2 | *H. sexstylosa* var. *ovata* |
| D | *H. sexstylosa* var. *ovata* |
| E | *H. sexstylosa* var. *ovata* |
| F | *H. sexstylosa* var. *ovata* |
| 3 | *H. sexstylosa* |
| 7 | *H. sexstylosa* |
| A | *H. sexstylosa* |
| C | *H. sexstylosa* |
| 5 | *H. angustifolia* |
| 6 | *H. angustifolia* |
| 8 | *H. populnea* "Poor Knights" |
| J | *H. populnea* "Poor Knights" |
| 9 | *H.* "tararua" |
| O | *H.* "tararua" |
| G | *H. populnea* |
| I | *H. populnea* |
| K | *H. glabrata* |
| L | *H. glabrata* |
| M | *H. lyallii* |
| N | *H. lyallii* |
| * | Group centroids. |

very low percentage of correctly classified spines. The second group, trees A and C on the scattergram, is represented by more clustered points closer to the perimeter of the graph. This group, while still not well classified, records a considerably higher number of correctly classified spines than the first group (Table 11). With the largest number of trees represented in this analysis, *H. sexstylosa* and *H. sexstylosa* var. *ovata* were the most misclassified taxa.

Notably the two trees of *H. lyallii* are separated from each other on the scattergram. However lying between and merging with the *H. lyallii* are the two trees of *H. glabrata*. These two taxa both merge with the remaining taxa.

This analysis offered more options of classification, which in turn altered the percentage of correctly classified spines. For example while *H* "tararua" recorded 83% of its spines to be correctly classified in the first discriminant analysis, it reached only 67% and 72% correctly classified in this analysis (Table 11). To analyse further the effect of the sample size and number of options on level of correct classification, two more analyses were performed.

### 3.3.4 Discriminant Analysis of the *H. Sexstylosa* Group Using Nine Trees

The third discriminant analysis looked at the nine trees of *H. sexstylosa* and *H. sexstylosa* var. *ovata*. With only these trees to classify into two groups, corresponding to taxa, the percentage of correctly classified spines was

Table 11 : Predicted group membership of spines for individual trees of *H. populnea* et al. study. Predicted membership of closest taxon is also listed.

| Taxon and Actual Tree No. | Scattergram Symbol | Predicted Tree No. (%) 1 | 2 | 3 | 4 | 5 | Total | Closest Taxon | Other Taxa |
|---|---|---|---|---|---|---|---|---|---|
| *H. sexstylosa* var. *ovata* | | | | | | | | *H. sexstylosa* | |
| 1 | 1 | 22.2 | 11.1 | 0 | 11.1 | 0 | 44.4 | 16.7 | 38.9 |
| 2 | 2 | 11.1 | 27.8 | 0 | 5.6 | 0 | 44.5 | 27.8 | 27.7 |
| 3 | D | 0 | 0 | 66.7 | 0 | 0 | 66.7 | 5.6 | 27.7 |
| 4 | E | 0 | 0 | 0 | 44.4 | 0 | 44.4 | 5.6 | 50.0 |
| 5 | F | 0 | 0 | 0 | 0 | 44.4 | 44.4 | 22.3 | 33.2 |
| *H. sexstylosa* | | | | | | | | *H. sexstylosa* var. *ovata* | |
| 1 | 3 | 38.9 | 0 | 0 | 0 | | 38.9 | 0 | 61.1 |
| 2 | 7 | 11.1 | 22.2 | 0 | 0 | | 33.3 | 33.3 | 33.4 |
| 3 | A | 0 | 0 | 50.0 | 5.6 | | 55.6 | 22.2 | 22.2 |
| 4 | C | 0 | 5.6 | 5.6 | 55.6 | | 66.8 | 16.7 | 16.5 |
| *H. glabrata* | | | | | | | | *H. lyallii* | |
| 1 | K | 61.1 | 0 | | | | 61.1 | 5.6 | 33.3 |
| 2 | L | 5.6 | 61.1 | | | | 66.7 | 0 | 33.3 |
| *H. lyallii* | | | | | | | | *H. glabrata* | |
| 1 | M | 66.7 | 0 | | | | 66.7 | 0 | 33.3 |
| 2 | N | 0 | 83.3 | | | | 83.3 | 0 | 16.7 |
| *H. populnea* | | | | | | | | *H. populnea* "Poor Knights" | |
| 1 | G | 50.0 | 0 | | | | 50.0 | 0 | 50.0 |
| 2 | I | 0 | 83.3 | | | | 83.3 | 0 | 16.7 |
| *H. populnea* "Poor Knights" | | | | | | | | *H. populnea* | |
| 1 | 8 | 77.8 | 5.6 | | | | 83.4 | 0 | 16.6 |
| 2 | J | 0 | 72.2 | | | | 72.2 | 5.6 | 22.2 |
| *H. angustifolia* | | | | | | | | | |
| 1 | 5 | 88.9 | 0 | | | | 88.9 | - | 11.1 |
| 2 | 6 | 0 | 38.9 | | | | 38.9 | - | 61.1 |
| *H.* "tararua" | | | | | | | | | |
| 1 | 9 | 72.2 | 16.7 | | | | 88.9 | - | 11.1 |
| 2 | 0 | 16.7 | 66.7 | | | | 83.4 | - | 16.6 |

59% in total (Table 12). Therefore with fewer classes available to group the trees into, a much higher percentage of correctly classified spines was computed than in the discriminant analysis of all taxa (Section 3.2.2). While the histogram (Figure 27) showed a considerable overlap between the two taxa, it was difficult to assign individual trees to the points on the graph, as had been possible in the initial investigation of *H. glabrata* and *H. lyallii* (Section 3.1).

Table 12 : Predicted group membership for the nine trees of *H. sexstylosa* and *H. sexstylosa* var. *ovata*

| Actual Group | Predicted Group | |
|---|---|---|
| | *H. sexstylosa* | *H. sexstylosa* var. *ovata* |
| *H. sexstylosa* | 58.3% | 41.7% |
| *H. sexstylosa* var *ovata* | 40.0% | 60.0% |

Total percent of spines correctly classified = 59.26%

### 3.3.5 Discriminant Analysis of the *H. sexstylosa* Group Using Four Trees

The fourth discriminant analysis looked only at two trees each of *H. sexstylosa* and *H. sexstylosa* var. *ovata*, for classification into two groups corresponding to taxa. This produced a total figure of 78% of spines correctly classified (Table 13). While the points representing both taxa are widely scattered on the histogram (Figure 28), clustering was more evident than in the previous analysis.

Figure 27. Frequency of Discriminant Scores for four trees of H. sexstylosa and five of H. sexstylosa var. ovata.

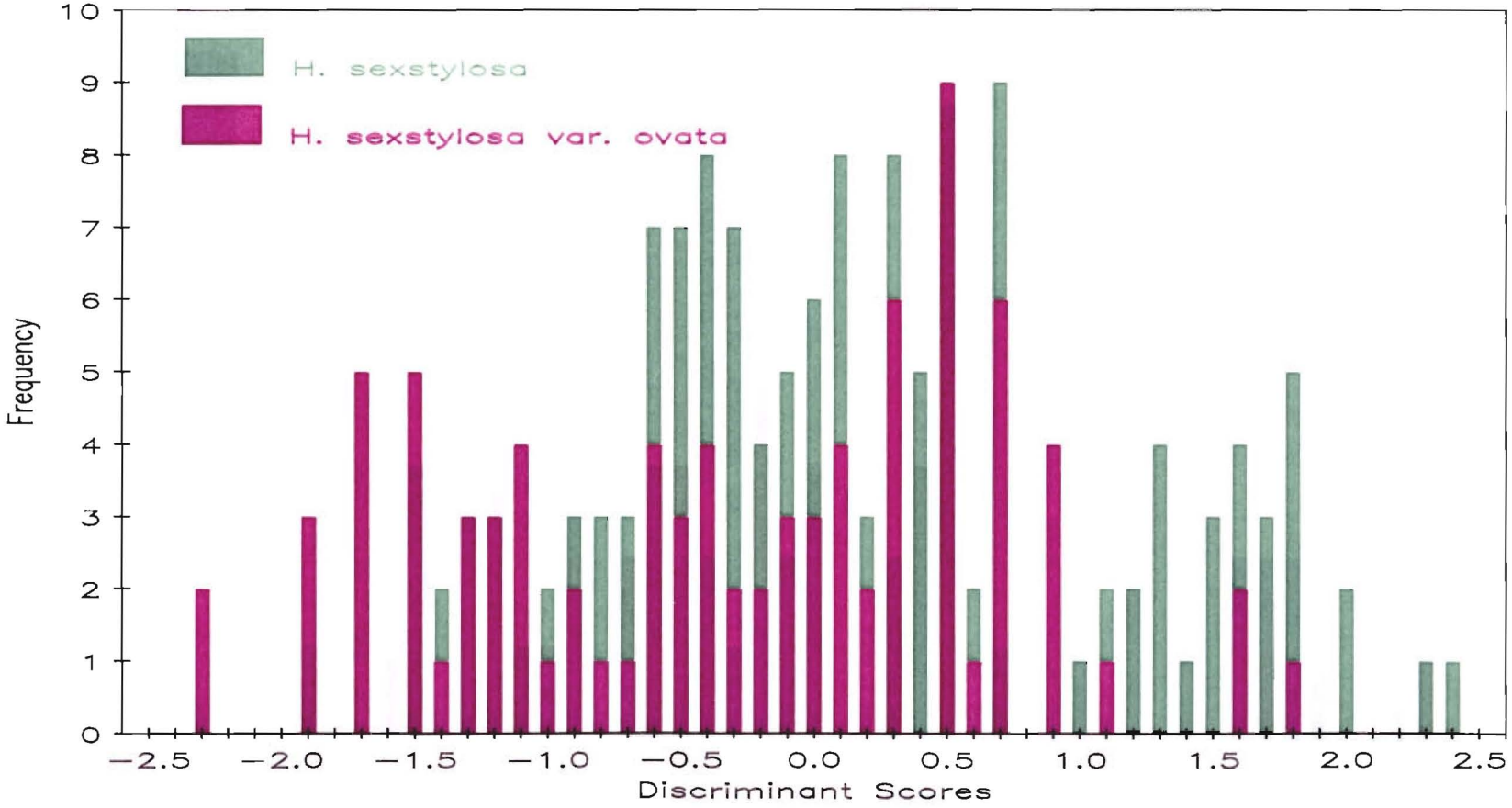


Figure 28. Frequency of Discriminant Scores for two trees each of H. sexstylosa and H. sexstylosa var. ovata.

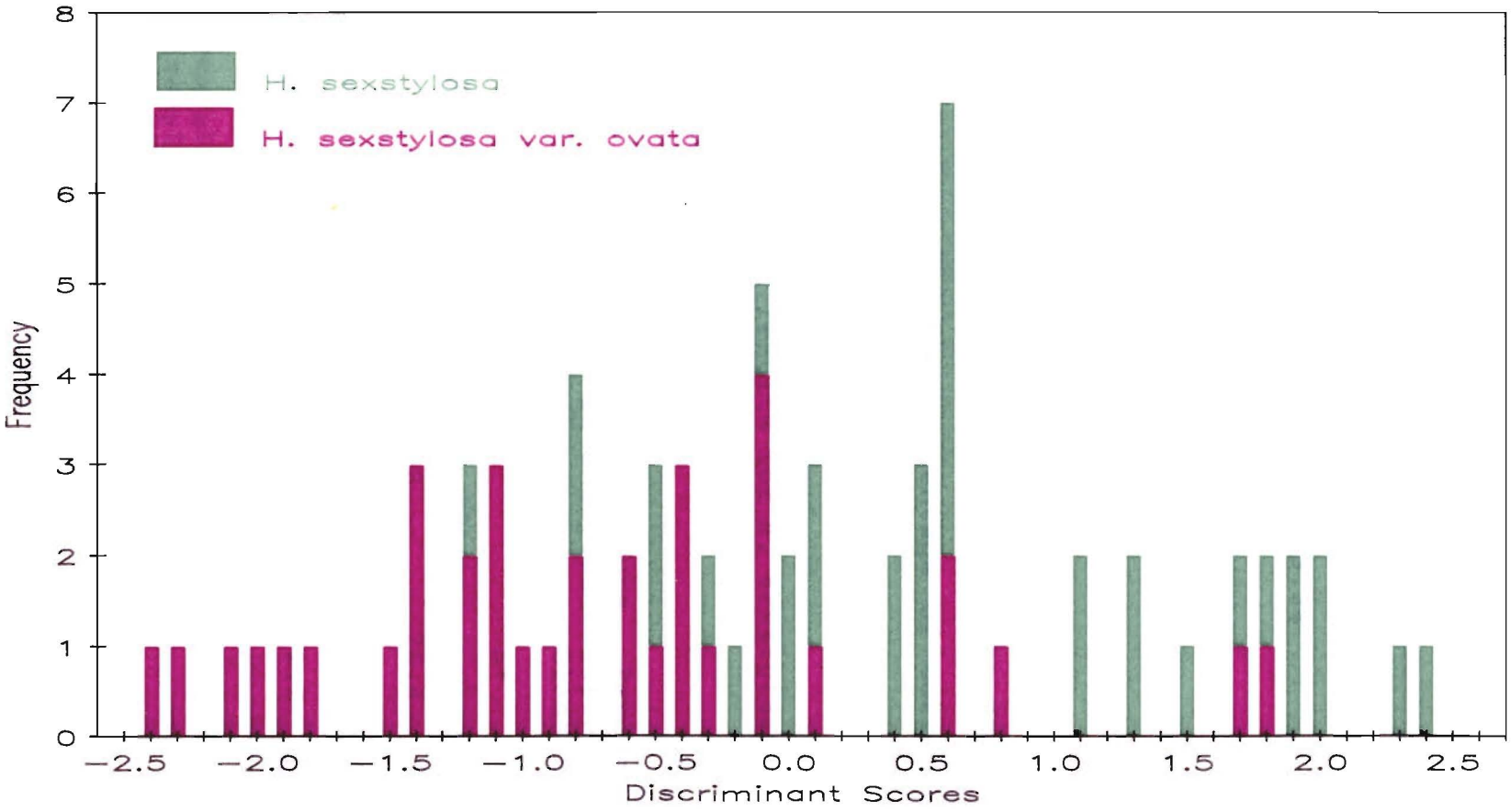


Table 13 : Predicted group membership for four trees of *H. sexstylosa* and *H. sexstylosa* var. *ovata*

| Actual Group | Predicted Group | |
|---|---|---|
| | *H. sexstylosa* | *H. sexstylosa* var. *ovata* |
| *H. sexstylosa* | 72.2% | 27.8% |
| *H. sexstylosa* var. *ovata* | 16.7% | 83.3% |

Total percent of spines correctly classified = 77.78%

Consequently when fewer groups are available for the classification, and when fewer trees per group are used in the analysis, the greater is the percentage of correctly classified spines (Table 14).

Table 14 : Comparison of results for *H. sexstylosa* and *H. sexstylosa* var. *ovata* in three discriminant analyses

| Analysis Reference | Total trees in analysis | No. of S* and O* trees in analysis | | No. of groups in analysis | Percent Correctly Classified | |
|---|---|---|---|---|---|---|
| | | S* | O* | | S* | O* |
| 1 | 21 | 4 | 5 | 8 | 26.4 | 24.4 |
| 2 | 9 | 4 | 5 | 2 | 58.3 | 60.0 |
| 3 | 4 | 2 | 2 | 2 | 72.2 | 83.3 |

S* = *H. sexstylosa*
O* = *H. sexstylosa* var. *ovata*

1 = Discriminant analysis by taxa for the *H. populnea* et al. study
2 = Discriminant analysis of the *H. sexstylosa* group using nine trees
3 = Discriminant analysis of the *H. sexstylosa* group using four trees

# CHAPTER 4

# DISCUSSION AND CONCLUSIONS

## 4.1 DISCUSSION

The investigations into *H. glabrata* and *H. lyallii* found these two taxa to be inseparable based on pollen morphology. Even when the 24 trees were analysed as individuals no intra-taxon groupings, which may have correlated with environmental variables, could be distinguished. Therefore these results lend no support to the present classification of these two taxa as separate species. Equally the analysis of pollen from the entire genus found that none of the eight member taxa could be separated from each other using pollen morphological characteristics. The two taxa exhibiting pollen which was slightly 'different' from the norm, *H. populnea* 'Poor Knights' and *H.* 'tararua', could at best only be correctly classified by discriminant analysis 81% and 83% of the time respectively. In light of these findings the investigations and their results are discussed in more detail below, and compared with the findings of other authors.

Using analysis of variance in the initial investigation of *H. glabrata* and *H. lyallii* it was discovered that variation in the nine pollen characters was highly significant at the grain, anther and tree levels in both taxa. Therefore individual branches and flowers

within trees did not yield pollen exhibiting significant variation. The discriminant analysis that followed revealed extensive variation of the pollen at both grain and anther levels. This is demonstrated by the frequent classification of different spines on the same grain and different grains from the same anther into different taxa. This result is further emphasised by the discriminant analysis of all 24 trees of *H. glabrata* and *H. lyallii*, where intraspecific variation of pollen size, and spine size, shape and density is as great as interspecific variation.

As the variation in pollen characters is not confined to particular anthers, flowers or branches within individual trees, it is unlikely that variation is a result of resource allocation. Pollen variation within trees is most likely to be a response to environmental effects, while pollen variation between trees is likely to be the result of both genetic and environmental factors.

While studies of pollen variation are in their infancy in New Zealand, much research has been carried out overseas on pollen variation, within and between different taxonomic levels of flowering plants. Pollen grain size variation, well documented in the literature, is considered by many to be a direct result of environmental influences. Jones and Newell (1948) determined that environmental conditions had a pronounced effect on the size of the pollen grains from many of the grass species which they studied in Nebraska. Mikkelsen (1949) believes nutrition to be an important factor controlling pollen size in samples of *Pelargonium zonale*. Unfortunately neither

paper was specific regarding which environmental or nutritional causes affected pollen size. Ritchie Bell (1959) found both intra-clonal and intraspecific differences in the mean pollen grain size of tomato, petunia, dill and portulaca plants grown in various nutrient solutions. Unfortunately mean pollen size measurements from each plant did not show any definite pattern of variation for specific nutrient solutions. Ritchie Bell (1959) like other authors (Harris 1965a, Olsson 1975) found intraspecific variation of pollen size to be as great as, or greater than, interspecific variation. Muller (1979) tabulates some of the environmental factors known to cause variation in pollen grain size. While mineral nutrition is reported to be the most important factor influencing size, other factors such as light intensity, temperature, water and root crowding are also listed. Clearly all of these factors will interact seasonally to produce considerable variation in pollen size. Consequently pollen size alone is generally not regarded as a reliable character for delimiting taxa. Cain and Cain (1948) measured grain size of *Pinus echinata* pollen from 27 sites in the southeastern United States. Their results showed that pollen grain size of this pine can be very similar from sites hundreds of miles apart, and very different from closely situated sites. However their results also showed evidence for a geographic cline, with mean pollen grain size being consistently larger in the northeastern part of their sample area, and declining in size towards the southern part.

In the present study *H. glabrata* and *H. lyallii* have been shown to be inseparable using discriminant analysis based on their pollen morphological characteristics. For the major *H. glabrata-H. lyallii* analysis 72% of the spines were correctly classified into each of the two taxa, while only 41% of the spines were correctly classified when the 24 trees were analysed as individual groups. However if *H. glabrata* and *H. lyallii* are a single species exhibiting clinal variation, as suggested by A.P. Druce (pers. comm.) and H.D. Wilson (pers. comm.), they may also show a geographic cline of mean pollen grain size, as Cain and Cain (1948) found for *P. echinata*. To determine this however, many more measurements of pollen size would be required than are available from this study.

When classified into eight groups, corresponding to taxa, none of the taxa of the *H. populnea* et al. study could be accurately distinguished by pollen characters using discriminant analysis (Table 10). *H. sexstylosa* and *H. sexstylosa* var. *ovata*, sampled more extensively than the other taxa, were found to have less than 30% of their spines correctly classified. However when *H. sexstylosa* and *H. sexstylosa* var. *ovata* were analysed separately from the other taxa, in the 'four tree' and 'nine tree' analyses (Table 14) the percentage of correctly classified spines rose dramatically. Consequently the structure of the discriminant analysis, that is the number of trees per taxon and the number of taxa per analysis, significantly affects the percentage of correctly classified spines. Those analyses with a greater number of trees per taxon and a larger number of taxa per analysis will achieve more

accurate results than analyses with very few samples and groups. This suggests that had the other six taxa also been represented by four or five trees in this analysis then they too would have had many more spines misclassified.

It is interesting to note that those spines which are misclassified frequently do not classify into taxa which are regarded as their closest relative by other criteria (Section 1.1). For example Table 10 shows that only 12% of the misclassified spines of *H. sexstylosa* var. *ovata* are classified into *H. sexstylosa*, while the remaining 63% of the misclassified spines are grouped in with other taxa. Almost one third of all misclassified spines fall into taxa outside their related group. The discrimination of groups to tree level shows even more the variable nature of pollen between trees of the same taxon (Table 11). For example *H. populnea* is represented by one tree with 50% of its spines correctly classified, and by another with 83% of its spines correctly classified; such variability is not limited to this taxon. The variability of pollen between trees of the same taxon is also expressed by the number of misclassified spines from one tree which are classified into trees of other taxa, and not into the remaining tree(s) of that taxon.

While no distinct groupings exist between taxa or trees on Figures 25 and 26, *H. populnea* 'Poor Knights' and *H.* 'tararua' come the closest to forming individual groups. This is most evident on Figure 25 where both of these taxa form semi-distinct groups towards the periphery of the scattergram. The peripheral positions of these two taxa

on Figure 25 shows that their pollen is slightly 'different' from the norm, which is represented in the central area of the scattergram. This is reinforced by Figures 18 and 19 which show the pollen variants that may occur in these two taxa. Figure 26 exemplifies the similarity of pollen from all trees. On this scattergram the 21 trees are even more mixed, although a single tree each of *H. populnea* 'Poor Knights' and *H. lyallii* show partial separation from the remainder.

For each discriminant analysis the discriminant function was calculated from spine, grain, tree and taxon information entered into the computer program for each spine case. Consequently, as each spine with its relative information helped to formulate the discriminant function, many more correctly classified spines might have been expected in this analysis. Therefore had 'unknown' pollen grains been entered into the program, that is those which had not helped to shape the discriminant function, then the percentage of correctly classified spines from these grains would almost certainly have been much lower.

Varying degrees of success have been achieved when correlating pollen variation with particular taxonomic levels of both modern and fossil pollen. While pollen morphological characters could not separate the eight taxa of the *Hoheria* genus, discriminant analysis based on a number of measured pollen characteristics is still a powerful tool for the separation of similar taxa. Birks and Peglar (1980) measured morphological characters on modern pollen grains of *Picea glauca*, *P. mariana*, and *P. rubens*, and using discriminant analysis could correctly

classify 92% of the grains. They then measured the same morphological characters on fossil Quaternary *Picea* pollen grains of 'unknown' species origin. Again analysing the data by discriminant analysis, they achieved results which were well supported by macrofossil evidence. Clearly however, with such a large margin of error it was important to verify their results with other evidence.

As in *Hoheria*, variations in pollen morphology do not always comply with present taxonomic divisions. Díez, Valdés and Fernández (1986) found considerable pollen morphological variation within and between species from the family Boraginaceae, while Raj and Grafström (1984) distinguished ten pollen types from the ten genera of the family Chloanthaceae. The ten pollen types however were not correlated with the generic divisions. Ackerman and Williams (1980) could not define the tribe Neottieae (family Orchidaceae) using any pollen characteristics, as they found a continuum existed for each character state. This result is most comparable to the results described here for *Hoheria*, where characters also appear to vary along a continuum.

Some authors have used pollen to support changes to present taxonomic divisions. Raj and Grafström (1984) suggest the splitting of the genera *Lachnostachys* and *Pityrodia* in the Chloanthaceae, based on pollen morphology. Zavada (1983) found pollen morphology to support the idea of taxonomic elevation of the subfamilies Ulmoideae and Celtidoideae to family level. This elevation was initially proposed by Grudzinskaya (1967, in Zavada 1983) based on a number of gross morphological characteristics.

Modern pollen studies have also led to the identification of fossil pollen in many parts of the world. Cain and Cain (1948) have identified fossil species of *Pinus* in southeastern United States, Birks and Peglar (1980) *Picea* in eastern North America, Miyoshi (1983) *Castanopsis* in Japan and Gortemaker (1986) species of *Fagus* in the Netherlands. However for the comparison of modern reference material to Quaternary material one must assume that the modern reference material encompasses the intraspecific variation through the range of species concerned, and that evolution since the time of pollen deposition has not been significant (Birks and Peglar 1980).

While the present research has shown that the genus *Hoheria* can not be separated using pollen morphological characteristics, a continuing investigation of *Hoheria* pollen over a number of years may provide interesting data for studies of pollen diversity. As a result both environmental influences and plant processes could be monitored to determine their effects on pollen variation annually. Haase (1985) found that cold and wet summers tended to reduce flowering and seed production in *H. glabrata*; could such conditions also effect pollen size or diversity? A poor or profuse seed crop the previous year may affect the quantity and diversity of pollen the following summer. Haase (1985) also found *H. glabrata* trees situated at Goat Pass, Arthurs Pass National Park, showed a steady decline throughout the five year period he studied them. He determined environmental stresses to be "clearly responsible". As such stresses affect gross

morphology of the trees, may they also affect pollen quantity and variation? While the pollen of the eight taxa of *Hoheria* can not be fully differentiated, clearly there is much scope for the continual monitoring of representative trees and their environment to ascertain the nature and causes of pollen variation.

## 4.2 CONCLUSIONS

Discriminant analysis was unable to differentiate the eight taxa of the *Hoheria* genus using pollen morphological characteristics. The results show that all taxa are intermixed to a greater or lesser extent based on these pollen characteristics. From the two separate discriminant analyses of the *H. sexstylosa* group it is clear that the percentage of correctly classified spines is controlled by the number of trees per taxon and the number of taxa per analysis. Therefore the six taxa with only two trees each sampled in the *H. populnea* et al. study, produced greatly inflated estimates of correctly classified spines. This is also true for the major *H. glabrata-H. lyallii* analysis where 72% of the spines were correctly classified. Here the discriminant analysis offered only two options, corresponding to taxa, into which the 24 trees could be grouped. Therefore the percentage of correctly classified spines was much higher than if more taxa had been included in the analysis.

As the pollen grains analysed in this study are all of known species origin, entering 'unknowns' would have resulted in a much higher percentage of misclassified

spines. Therefore it follows that if pollen morphological characters cannot be used to separate modern *Hoheria* pollen grains, they cannot be used to separate fossil pollen.

As a result of this research significant variation was found in the pollen at grain, anther and tree levels. Generally the variation in morphology can not be grouped into distinct types. However, *H. populnea* 'Poor Knights' and *H.* 'tararua' both exhibit pollen that is slightly different from the norm. This results in a higher percentage of correctly classified spines for these two taxa. Had more samples been analysed for these taxa however, the number of correctly classified spines would most likely have significantly decreased, as happened with the *H. sexstylosa* group. Therefore pollen morphology is of no real value in the classification of the *Hoheria* genus, or for correlation with climatic or other environmental parameters. If a separation into individual taxa had been possible, this would have been achieved by discriminant analysis.

While a continuum appears to exist for each character state measured, the extent to which pollen varies in the genus *Hoheria* is itself interesting. With this knowledge the next step could be to select specific characters, such as pollen size or spine density, and measure these on pollen grains from selected trees of the eight taxa from localities around New Zealand. Mean calculations of these characters at each sample site could then be compared, to determine whether geographic variation for specific morphological characters exists for *Hoheria*. Environmental variables could also be monitored over a

number of consecutive years to determine their affects on pollen variation.

# REFERENCES

Ackerman, J.D.; Williams, N.H. 1980; Pollen morphology of the tribe Neottieae and its impact on the classification of the Orchidaceae. *Grana*, 19: 7-18.

Allan, H.H. 1926: Epharmonic response in certain New Zealand species and its bearing on taxonomic questions. *J. of Ecology*, 14: 72-91.

_____ 1961: Flora of New Zealand, Vol. 1, Wellington, Government Printer, 1085p.

Anderson, T.F. 1951: Techniques for the preservation of three dimensional structure in preparing specimens for the Electron Microscope. *Trans. N.Y. Acad. Sci.*, 13: 130-133.

Batchelor, C.L. 1971: Estimation of density from a sample of joint point and nearest-neighbour distances. *Ecology*, 52(4): 704-709.

_____ 1973: Estimating density and dispersion from truncated or unrestricted joint point-distance nearest-neighbour distances. *Proc. N.Z. Ecol. Soc.*, 20: 131-147.

_____ 1975: Probable limit of error of the point distance-neighbour distance estimate of density. *Proc. N.Z. Ecol. Soc.*, 22: 28-33.

Birks, H.J.B.; Peglar, S.M. 1980: Identification of *Picea* pollen of late Quaternary age in eastern North America: a numerical approach. *Can. J. Botany*, 58: 2043-2058.

Boyde, A.; Wood, C. 1969: Preparation of animal tissues for surface-scanning electron microscopy. *J. of Microscopy*, 90(3): 130-133.

Cain, S.A.; Cain, L.G. 1948: Size frequency characteristics of *Pinus echinata* pollen. *Botany Gazette*, 110; 325-330.

Chinnappa, C.C.; Warner, B.G. 1982: Pollen morphology in the genus *Coffea* (Rubiaceae). II Pollen polymorphism. *Grana*, 21: 29-37.

Christensen, P.B. 1986: Pollen morphological studies in the Malvaceae. *Grana*, 25: 95-117.

Clarke, G. 1978: Pollen morphology and generic relationships in the Valerianaceae. *Grana*, 17: 61-75.

Coetzee, J.; van der Schijff, H.P. 1979: Pollen morphology of South African Malvales: 1. Characteristics useful for keying and for numerical analysis. *J. Sth. Afr. Bot.*, 45(2): 93-126.

Díez, M.J.; Valdés, B.; Fernández, I. 1986: Pollen morphology of Spanish *Lithospermum* s.l. (Boraginaceae) and its taxonomic significance. *Grana*, 25: 171-176.

Eagle, A. 1975: Eagle's Trees and Shrubs of New Zealand in Colour. Auckland, Collins, 311p.

_____ 1982: Eagle's Trees and Shrubs of New Zealand: Second series. Auckland, Collins, 382p.

Erdtman, G. 1960: The acetolysis method, a revised description. *Svensk Botanisk Tidskrift*, BD54: 561-564.

Faegri, K.; Iversen, J. 1975: Textbook of Pollen Analysis. 3d ed. Oxford, Blackwell Scientific Pub. Ltd., 295p.

Gordon, A.B.; Prentice, I.C. 1977: Numerical methods in Quaternary Palaeoecology: 4. Separating mixtures of morphologically similar pollen taxa. *Review of Palaeobotany and Palynology*, 23: 359-372.

Haase, P. 1985: Ecological investigation of some subalpine trees and shrubs. Christchurch, University of Canterbury, 263p. (Thesis: Ph.D. Botany).

Harris, W.F. 1956a: Pollen characters in *Northofagus*, *N.Z Sci. and Tech.*, 37: 731-765.

_____ 1956b: Pollens of *Nothofagus*, *N.Z.J. Sci. and Tech.*,37: 635-638.

Hooker, J.D. 1853: Flora Novae Zelandiae. 1, London, Reeve, 312p.

Jones, M.D.; Newell, L.C. 1948: Size variability and identification of grass pollen. *J. of the Am. Soc. of Agronomy*, 40: 136-143.

Mack, R.N. 1971: Pollen size variation in some western North American pines as related to fossil pollen identification. *Northwest Science*, 45: 257-269.

McNeill, J.; Crompton, C.W. 1978: Pollen dimorphism in *Silene alba* (Caryophyllaceae). *Can. J. Botany*; 55: 1280-1286.

Mikkelsen, V.M. 1949: Has temperature any influence on pollen size? *Physiologia Plantarum*, 2: 323-324.

Miyoshi, N. 1983: Pollen morphology of the genus *Castanopsis* (Fagaceae) in Japan. *Grana*, 22: 19-21.

Muller, J. 1979: Form and function in Angiosperm pollen. *Ann. Missouri Bot. Gard.*, 66: 593-632.

Olsson, U. 1975: On the size and microstructure of pollen grains of *Quercus robur* and *Q. petraea* (Fagaceae). *Bot. Notiser*, 128; 256-263.

Pocknall, D. 1979: Studies on the pollen of New Zealand Gymnosperms. Christchurch, University of Canterbury, 278p. (Thesis: Ph.D. Botany).

Raj, B.; Grafström, E. 1984: A contribution to the pollen morphology of Chloanthaceae (Benth.) Hutch. *Grana*, 23: 139-156.

Ritchie Bell, C. 1959: Mineral nutrition and flower to flower pollen size variation. *Am. J. Botany*, 46(9): 621-624.

Sáenz de Rivas, C. 1979: Pollen morphology of Spanish Cistaceae. *Grana*, 18: 91-98.

Salmon, J.T. 1980: The Native Trees of New Zealand. Wellington, A.H. & A.W. Reed Ltd., 384p.

Sokal, R.R.; Rohlf, F.J. 1981: Biometry. 2d ed. San Francisco, W.H. Freeman and Co., 859p.

Ting, W.S. 1966: Determination of *Pinus* species by pollen statistics. *Univ. Calif. Publ. Geol. Sci.*, 58, 168p.

Zavada, M. 1983: Pollen morphology of Ulmaceae. *Grana*, 22: 23-30.

**Appendix I : Location of trees used in all *H. glabrata* and *H. lyallii* analyses.**

| Taxon | Tree No. | Location | Coordinates |
|---|---|---|---|
| *H. glabrata* | 1 | Greyneys Flat | 170°35'E 42°59'S |
| | 2 | Greyneys Flat | 171°35'E 42°59'S |
| | 3 | 150 m north Otira township | 171°34'E 42°50'S |
| | 4 | Bottle Flat, Otira Gorge | 171°34'E 42°53'S |
| | 5 | Klondyke Corner | 171°35'E 43°00'S |
| | 6 | South side Arthurs Pass township | 171°34'E 42°57'S |
| | 7 | Foot of Temple Basin Road | 171°34'E 42°55'S |
| | 8 | Foot of Temple Basin Road | 171°34'E 42°55'S |
| | 9 | 1 km north Dobson Memorial | 171°33'E 42°54'S |
| | 10 | Pegleg Creek | 171°34'E 42°54'S |
| | 11 | 12 km north Otira township | 171°34'E 42°49'S |
| | 12 | Homer Tunnel | 168°57'E 44°47'S |
| *H. lyallii* | 1 | Ribbonwood Stream | 171°46'E 43°05'S |
| | 2 | Lake Selfe | 171°31'E 43°14'S |
| | 3 | Lake Selfe | 171°31'E 43°14'S |
| | 4 | Awa Awa Rata Reserve, Mt Hutt | 171°35'E 43°29'S |
| | 5 | Awa Awa Rata Reserve, Mt Hutt | 171°35'E 43°29'S |
| | 6 | Awa Awa Rata Reserve, Mt Hutt | 171°35'E 43°29'S |
| | 7 | Awa Awa Rata Reserve, Mt Hutt | 171°35'E 43°29'S |
| | 8 | Pylon Gully | 171°42'E 43°02'S |
| | 9 | Pylon Gully | 171°42'E 43°03'S |
| | 10 | Pylon Gully | 171°42'E 43°02'S |
| | 11 | Pylon Gully | 171°42'E 43°02'S |
| | 12 | Pylon Gully | 171°42'E 43°02'S |

Appendix II : Herbarium samples used in the *H. populnea* et al. study.

| Taxon | Tree No. | Location | Collector | Herbarium No. |
|---|---|---|---|---|
| *H. sexstylosa* var. *ovata* | 1 | Onamalutu, Richmond Ra., Marlborough | A.P. Druce | CHR387051 |
| | 2 | Takaka Hill, N.W. Nelson | A.P. Druce | - |
| | 3 | Lower Buller River Westland | L.J. Metcalf | No. 200497 |
| | 4 | Takaka Hill Summit N.W. Nelson | A.P. Druce | CHR273478 |
| | 5 | Onamalutu, Richmond Ra., Marlborough | | CHR387058 |
| *H. sexstylosa* | 1 | 25 km NE Wanganui | A.P. Druce | CHR221328 |
| | 2 | Brocketts Strm, Western Lake Rd., Wairarapa | R. Mason | No. 65656 |
| | 3 | Kitchena Park | - | No. 11711 |
| | 4 | Reserve, Western Lake, Wairarapa | | CHR307016 |
| *H. populnea* | 1 | Taheke-Horeke Rd., Hokianga County | R.C. Cooper | AK127274 |
| | 2 | Waitakere Ra., Scenic Drive | A.E. Orchard | AK145096 |
| *H. populnea* "Poor Knights" | | | | |
| | 1 | Poor Knights Is. | A.P. Druce | CHR386856 |
| | 2 | Poor Knights Is. | A.T. Pycroft | AK102953 |
| *Hoheria* "tararua" | 1 | Mt Mathews, Rimutaka Ra. | A.P. Druce | CHR197299 |
| | 2 | 6 km SSW of Ketahuna | A.P. Druce | No.197296 |
| *H. angustifolia* | 1 | Kaituna Valley Canterbury | D.H. Smith | CHR229247 |
| | 2 | Kaituna Valley Canterbury | E.J. Beuzenberg & B.E. Groves | No.200489 |

# GLOSSARY OF MORPHOLOGICAL TERMS

Ektexine : the outer layer of the exine, usually sculptured.

Exine : the outer, resistant, non-living layer of the pollen cell wall.

Verrucae : small wart-like elements found on the outer surface of the exine.